Pergamon

Tetrahedron, Vol. 53, No. 39, pp. 13183-13220, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00477-8

TETRAHEDRON REPORT NUMBER 427

Selective Oxidations Catalyzed By Peroxidases

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Contents

1. INTRODUCTION

General introduction

The current interest in catalytic oxidative transformations has been stimulated by two major environmental issues. The first one is the need for replacement of oxidations which use stoichiometric amounts of heavy metal salts by cleaner catalytic alternatives using hydrogen peroxide or oxygen as the oxidant. The second major issue is the increasing demand for high chemo-, regio- or stereoselectivity in chemical reactions in order to improve chemical yields, to minimize waste streams and to avoid enantiomeric ballast. Redox enzymes are potentially suitable for meeting these two goals. Redox enzymes (*i.e.* oxidoreductases) can be classified into the following categories according to the oxidant they use and the reactions they catalyze:

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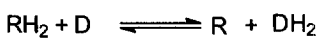
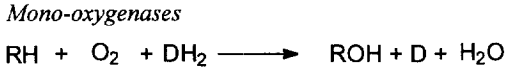
Dehydrogenases/Reductases



Oxidases

Oxygenases

Mono-oxygenases



Dioxygenases

Peroxidases

D,DH_2 = cosubstrate

Dehydrogenases are the most extensively studied redox enzymes for synthetic applications. However, these enzymes require stoichiometric quantities of expensive cosubstrates such as NADH; the regeneration of these is an economic necessity in large scale applications. The enantioselective reduction of α-keto acids with continuous regeneration of the NADH cofactor[1] constitutes an example. Mono-oxygenases, which mediate the introduction of an oxygen atom into the substrate, also have a cosubstrate requirement. Furthermore, they are relatively unstable and difficult to isolate. Hence, mono-oxygenases are often used in whole cell systems which are, however, prone to competing reactions which can lower the enantio-selectivity and yield of a reaction. Processes using dioxygenases are, like mono-oxygenase-mediated processes, carried out as precursor fermentations and have the same limitations. Oxidases have as a drawback that they generally exhibit a narrow substrate range which limits their synthetic utility. They are often used in biosensors for selective detection of components like glucose and cholesterol. Peroxidases, on the other hand, are interesting catalysts for performing selective oxidations using clean oxidants. They catalyze oxidative transformations of organic substrates with a peroxide, usually hydrogen peroxide. Unlike mono-oxygenases and dehydrogenases, peroxidases have no requirement for expensive cosubstrates. Moreover, most peroxidases are relatively stable extracellular enzymes that can accommodate a broad range of substrates in a diversity of reactions. These include reactions characteristic of both dehydrogenases as well as mono-oxygenases.

Peroxidases

Peroxidases are ubiquitous in nature[2-9]. Some peroxidases like ascorbate peroxidase act as H_2O_2 scavengers. Others such as horseradish peroxidase, catalyze free radical oligomerizations and polymerizations

of electron rich aromatics. Lignin peroxidase catalyzes the oxidative degradation of lignin. Although the exact role of haloperoxidases (peroxidases which can halogenate organic substrates, *vide infra*) is not yet clear the halometabolites they produce probably play an important role in the defense system of organisms.

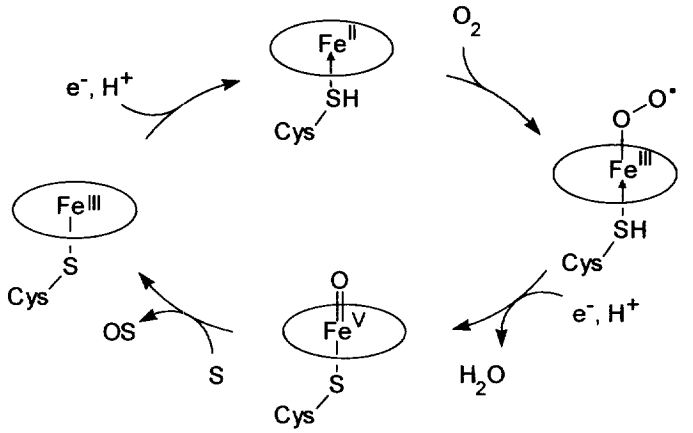


Fig. 1. Reaction cycle of monoxygenases

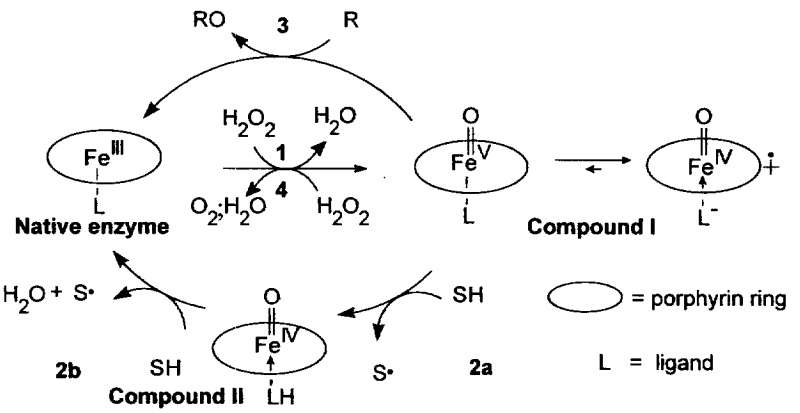


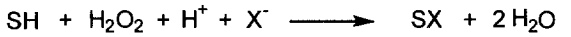
Fig. 2. Reaction cycle of peroxidases

Many peroxidases contain heme (iron(III) protoporphyrin IX) as the prosthetic group, although some peroxidases have different catalytic centers (*vide infra*). During catalysis heme peroxidases are oxidized by a peroxide to a (formally) iron(V) oxo species which is predominantly present as an iron(IV) oxo porphyrin radical cation species in most peroxidases (Fig. 2). This so called compound I is comparable to the iron(V) oxo intermediate in the mono-oxygenase catalytic cycle (Fig. 1). Instead of molecular oxygen and a cosubstrate which transfers 2 hydrogen atoms, peroxidases can directly form the iron(V) oxo species from native enzyme and hydrogen peroxide (pathway 1 in Fig. 2). Compound I can be reduced to native enzyme by several

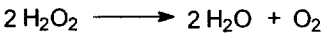
mechanisms depending on the type of reaction. Peroxidase reactions can be divided into the following four categories:

1. *Oxidative dehydrogenation* (classical peroxidase reaction, pathways 2a and 2b in Fig. 2)

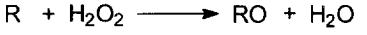
2. *Oxidative halogenation* (pathway 3 in 2nd, R = X^-)



3. *H_2O_2 disproportionation* (catalase reaction, pathway 4 in 2nd)



4. *Oxygen transfer reactions* (pathway 3 in 2nd, R = organic substrate)



Reactions

Oxidative dehydrogenation. This type of reaction is mainly restricted to heme peroxidases. Oxidative dehydrogenation involves one-electron transfer processes with radical cations and radicals as intermediates (pathways 2a and 2b in Fig. 2). The iron(V) oxo species is reduced to native enzyme via two one-electron transfers. The intermediate iron(IV) oxo species which is one oxidation equivalent above the native enzyme is called compound II. Peroxidases catalyze a variety of one-electron oxidations of electron-rich aromatics resulting in inter- or intra-molecular radical coupling products. This type of reaction is called the classical peroxidase reaction as it was the first type of reaction of peroxidases discovered. Examples of such reactions are shown in Fig. 3 and Fig. 4.

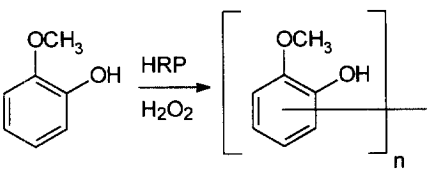


Fig. 3. Oxidation of guaiacol, standard assay[10]

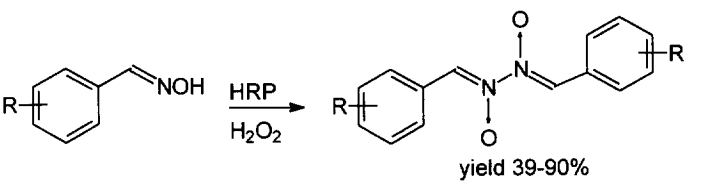


Fig. 4. Aldoxime oxidation to iminoxy dimers by HRP[11]

A useful classical peroxidase reaction which deserves to be mentioned is the peroxidase catalyzed polymerization of phenols and anilines (Fig. 5) under mild conditions[12-15].

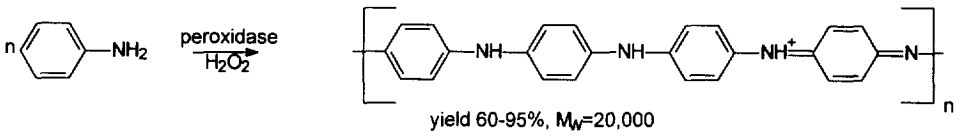


Fig. 5. Polymerization of aniline by a peroxidase[12]

N- and *O*-dealkylation are a special case of electron transfer processes catalyzed by peroxidases. These are interesting reactions from a synthetic point of view because oxidative dealkylation is difficult to perform in organic chemistry. Dealkylation of tertiary amines, which is relatively facile, requires stoichiometric amounts of oxidants, like permanganate, chlorine dioxide or alkaline potassium ferricyanide. Dealkylations of secondary amines are more difficult to perform and often harsh reaction conditions are necessary. Heme proteins, peroxidases included, can catalyze the dealkylation of hetero-atoms[16]. An example is shown in Fig. 6. As this review is primarily concerned with the use of peroxidases for selective oxygen transfer processes we will not deal in detail with the classical peroxidation reaction.

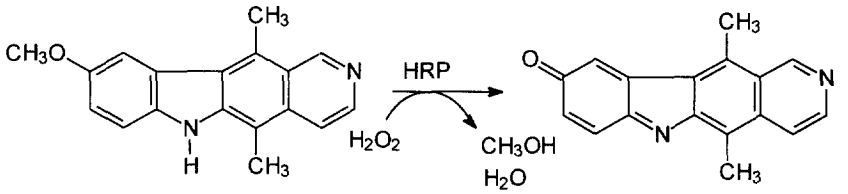


Fig. 6. *O*-demethylation of 9-methoxyellipticine catalyzed by HRP[17]

Oxidative halogenation. A special class of peroxidases comprises the so-called haloperoxidases which mediate the halogenation of organic substrates. Once thought to be rare -until 40 years ago only 3 examples were known[4]- it has since then become clear that haloperoxidases occur widely in nature. Since the 1970's over 100 sources of haloperoxidases have been discovered[7]. Oxidative halogenation is not limited to heme peroxidases, but is also catalyzed by vanadium haloperoxidases and other non-heme haloperoxidases (*vide infra*). Oxidative halogenation is believed to proceed via an active halide species. In the case of vanadium and heme peroxidases (pathway 3 in Fig. 2, R= halide) this active species is probably hypohalite[8]. Metal-free haloperoxidases are also known which presumably involve the formation of a peroxycarboxylic acid intermediate analogous to the lipase-catalyzed formation of peroxycarboxylic acids from hydrogen peroxide and a carboxylic acid (*vide infra*). One of the most versatile peroxidases is a chloroperoxidase from *Caldariomyces fumago* (CPO) which was first isolated in 1961 by Hager[18]. In vivo CPO is involved in the production of the chlorometabolite caldariomycin (1,1-dichloro-2,5-dihydroxy cyclopentane, Fig. 7). A standard assay for CPO involves the chlorination of monochlorodimedone to dichlorodimedone (Fig. 8)[19].

Peroxidase-catalyzed oxidative halogenation reactions are generally non-selective and halogenation of the substrate is believed to take place outside the active site. There is one example, however in which regioselectivity is observed: the metal-free chloroperoxidase from *Pseudomonas pyrrocinia* selectively converts indole to 7-chloro-indole[20]. We note that in order to exclude that this reaction proceeds via an enzyme-halide intermediate rather than via a peroxyacetic acid intermediate, a blank reaction with peroxyacetic acid and chloride should be carried out. Oxidative halogenation will not be further considered in detail in this paper. There are several reviews concerning halogenation reactions catalyzed by peroxidases[7-9].

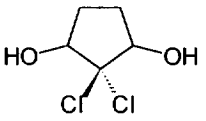


Fig. 7. Caldariomycin

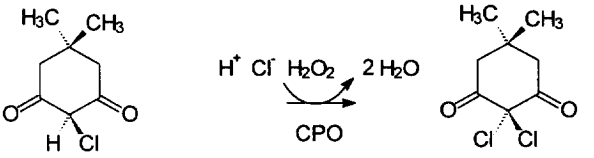


Fig. 8. Halogenation of monochlorodimedone by CPO (standard assay)

H_2O_2-disproportionation. Hydrogen peroxide decomposition to water and oxygen is catalyzed by some peroxidases, either directly[21,22] (via pathway 4 in Fig. 2) or by hypohalous acid production which subsequently reacts with hydrogen peroxide to produce singlet oxygen[23,24]. Especially CPO exhibits substantial catalase activity when hydrogen peroxide is the only reductant present in the reaction mixture[22]. During the last decade a new class of peroxidase related enzymes has been discovered, the catalase-peroxidases. They exhibit in addition to catalase activity also a substantial classical peroxidase activity[25].

Oxygen-transfer reactions. Selective oxygen transfer is the fourth type of reaction which is catalyzed by per-oxidases (pathway 3 in Fig. 2, R=organic substrate). From a synthetic point of view they are the most interesting oxidative transformations catalyzed by peroxidases. The transformations are comparable to those catalyzed by mono-oxygenases. Hence, many oxygen transfer reactions described in this review are not only catalyzed by peroxidases but also by mono-oxygenases. The oxygenase-type reactions of peroxidases can be divided into the following categories:

1. *Hetero-atom oxidation:*
   S-oxidation
   N-oxidation
2. *Epoxidation*
3. *CH bond oxidation:*
   Benzylic/allylic oxidation
   Alcohol oxidation
   Indole oxidation

This review is focused on synthetic and mechanistic aspects of selective oxygen transfer reactions catalyzed by peroxidases. Potential commercial applications and the question of enzyme deactivation are also discussed.

2. OXYGEN-TRANSFER REACTIONS

Hetero-atom oxidation

Sulfur oxidation. Chiral sulfoxides are useful auxiliaries in asymmetric synthesis[26,27]. Furthermore, certain sulfoxides containing a chiral sulfinyl group possess interesting biological properties. There are several methods for their preparation, involving both stoichiometric and catalytic oxidation of the corresponding sulfides. Examples include chemical methods[28], biomimetic approaches[29] and enzymatic approaches[30]. There are several recent reviews on the preparation of chiral sulfoxides[26,27,30-32].

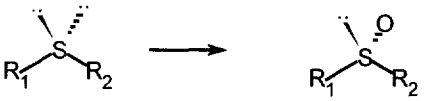


Fig. 9. Chiral sulfoxide formation from oxidation of the sulfide

In the last decade it has been shown that peroxidases are also capable of catalyzing sulfoxidation reactions[33-36]. The first enantioselective sulfoxidation using CPO was described by Kobayashi[37] (methyl *p*-methyl-phenyl sulfide yielded *R*-sulfoxide in 13% *ee*). The fairly low enantioselectivity in this report was probably due to substantial non-enzymatic oxidation caused by the high concentration of hydrogen peroxide used. More recently, CPO-catalyzed enantioselective sulfoxidations were investigated in more detail by Colonna *et al.*[38,39].

The best results were obtained with H_2O_2 as the oxidant. Substantial uncatalyzed oxidation of the sulfides (10-30%) was observed in blank reactions without catalyst, therefore many of the obtained sulfoxides in the enzymatic procedure were not completely enantiopure. When we undertook a study to investigate the influence of *tert*-butyl alcohol on the reactivity and selectivity of CPO for sulfoxidation we found essentially no uncatalyzed reaction in water and enantiopure sulfoxides were obtained in water as well as in *tert*-butyl alcohol/water mixtures[40] (Table 1). The reason for the higher blank reactions of Colonna *et al.* might well be the presence of trace amounts of metal oxides which are known to catalyze the oxidation of sulfides to the corresponding sulfoxides[32].

Table 1. Oxidation of sulfides by CPO and H_2O_2[40,a]

Sulfide		*tert*-butyl alcohol/buffer (50:50, v/v)		buffer	
R_1	R_2	conversion (%)	*ee* (%)	conversion (%)	*ee* (%)
C_6H_5	CH_3	73	99	100	99
C_6H_5	CH_2CH_3	52	99	83	99
C_6H_5	$CH_2CH_2CH_3$	1	60	3	27
p-CH_3-C_6H_4	CH_3	66	99	83	99
p-OCH_3-C_6H_4	CH_3	50	99	53	99
m-OCH_3-C_6H_4	CH_3	19	99	37	99
o-OCH_3-C_6H_4	CH_3	2	99	3	99
p-NO_2-C_6H_4	CH_3	17	99	19	99
p-Cl-C_6H_4	CH_3	73	99	78	99
m-Cl-C_6H_4	CH_3	50	99	59	99
p-Br-C_6H_4	CH_3	46	99	15	99
m-Br-C_6H_4	CH_3	22	99	11	99
p-Cl-C_6H_4	CH_2CH_3	49	99	33	99
	CH_3	91	99	100	99
	CH_3	80	99	100	99

a. 50 mM Sulfide, 25 ml solvent, 610 U CPO, 1 eq. H_2O_2/ 2h.

In a subsequent study the group of Colonna investigated the influence of chloride on the sulfoxidation by CPO[41]. Although the reaction rate increased, the enantiomeric excess of the sulfoxides decreased suggesting that oxidation partially proceeded via the intermediate formation of hypochlorite. Fu *et al.* investigated the chloroperoxidase catalyzed oxidation of *p*-substituted alkyl phenyl sulfides by hydrogen peroxide or racemic alkyl hydroperoxides as the oxidant in aqueous buffer[42]. Slow addition of the hydrogen peroxide to the reaction mixture afforded nearly enantiopure sulfoxides (*ee* = 97-99%). When racemic alkyl hydroperoxides were used as the oxidant, optically active alcohols and alkyl hydroperoxides were obtained (Fig. 10, *ee* up to 89%).

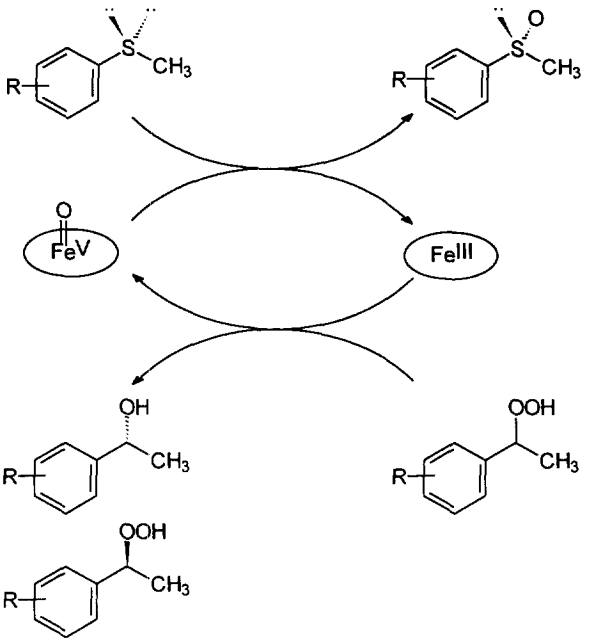


Fig. 10. Concomitant oxidation of sulfides and reduction of hydroperoxides catalyzed by CPO[42]

Oxidation of sulfoxide to sulfone was reported to be catalyzed by CPO for dimethylsulfoxide to dimethylsulfone[43]. However, overoxidation of the obtained sulfoxides (Table 1) to the sulfones was not observed in the CPO-catalyzed enantioselective sulfoxidations.

Table 2. Comparison of peroxidases for the sulfoxidation of methyl phenyl sulfide

Enzyme	reaction time (min.)	Yield (%)	*ee* (%)	TON[a]
CPO[39]	60	100	98 [*R*]	6.3×10^4
HRP[44]	60	95	46 [*S*]	29
LPO[49]	105	40	52 [*R*]	57
CcP[47]	not reported	not reported	2 [*S*]	<300
MP-11[48]	45	45	3 [*S*]	3

a. TON (Turnover number) = mole of product produced per mole of enzyme used

Several other heme-peroxidases were found to catalyze the enantioselective sulfoxidation of alkyl aryl sulfides. These include horseradish peroxidase[33,44-46] (HRP), cytochrome *c* peroxidase[47] (CcP), micro-

peroxidase[48] (MP) and lactoperoxidase[33,34,49] (LPO). However, their turnover numbers (TON) as well as enantioselectivities (*ee*) were much lower than those observed with CPO (Table 2).

The differences in reactivity and enantioselectivity of the peroxidases can be explained by the difference in the environment of the active site. Especially heme peroxidases in which the heme-iron is less accessible give a lower selectivity and reactivity (*vide infra*). In order to facilitate direct oxygen transfer, the active site of several heme peroxidases was enlarged using site-directed mutagenesis. Several mutants of HRP and CcP were made and the influence of the mutation on reaction rate and enantiomeric excess of thioether sulfoxidation was determined[47,50,51,52]. Replacement of the bulky tryptophan-51 in CcP by an alanine still resulted in nearly racemic sulfoxide (*ee* = 10%) although nearly all the oxygen in the sulfoxide was derived from hydrogen peroxide (98%). Modifications of HRP were made at two amino acids in the distal pocket of HRP, His-42 and Phe-41. Phe-41 was replaced by the amino acids leucine or threonine which are of intermediate size and by the small amino acid alanine. Also the distal histidine was replaced by alanine and by valine. The sulfoxidation of alkyl aryl sulfides proceeded faster for the mutants than for the native enzyme (Table 3). Moreover, the enantioselectivity in the case of F41L was increased. The results indicated that the alkyl chain of the alkyl aryl sulfides binds in a restricted site of HRP, and the reaction rate can be increased by replacing Phe-41 or His-42 with smaller amino acids. Furthermore, the stereospecificity of the reaction was shown be dependent on the size as well as on the polarity of the amino acid on position 41.

A peroxidase-related soybean oxidoreductase[53] is also capable of performing enantioselective sulfoxidations. This enzyme can use cumene hydroperoxide or long chain unsaturated alkyl hydroperoxides such as 13-hydroperoxylinoleic acid as the oxidant but does not catalyze the classical peroxidase reaction. High enantiomeric excesses to the *S*-sulfoxide were reported (90% *ee* for methyl *p*-tolyl sulfoxide).

Table 3. Effect of site directed mutagenesis of HRP on rate and enantioselectivity[52a,b]

Sulfide R_1-S-R_2		native HRP rate (nmol s^{-1} μmol^{-1})	native HRP *ee* (%)	F41L rate (nmol s^{-1} μmol^{-1})	F41L *ee* (%)	F41T rate (nmol s^{-1} μmol^{-1})	F41T *ee* (%)
R_1	R_2						
C_6H_5	CH_3	56.4	77	166.7	97	110.7	10
C_6H_5	cyclopropyl	8.5	7	94.6	70	173.0	5 (*R*)

a. The absolute stereochemistry of the dominant isomer is *S* except where indicated.
b. Rates were calculated from v_{max} and K_m at a sulfide concentration of 5 mM.

N-oxidation. A few examples of peroxidase-catalyzed selective *N*-oxidations have been described. For example Corbett et al. reported in 1979 the oxidation of 4-chloroaniline to 4-chloronitrosobenzene catalyzed by

CPO with H_2O_2 as the oxidant[54]. Later it was shown that other aryl amines could also be oxidized to the corresponding nitroso compounds[55-58]. The reaction involves direct oxygen transfer from the iron(V) oxo complex to the aniline and proceeds via the hydroxylamine which is further oxidized by a second iron(V) oxo species to the nitroso compound (Fig. 11).

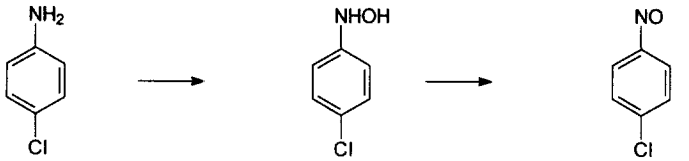


Fig. 11. Oxidation of anilines by CPO

A peroxidase-related enzyme from pea seedlings is also capable of mediating the oxidation of arylamines to the corresponding nitroso compounds[58-60]. When HRP was used as catalyst for oxidation of aniline-derivatives a complex mixture of high molecular weight coloured products was obtained[4,54,61]. Recently Kalliney and Zaks[62] discovered that HRP can selectively catalyze the oxidation of the hydroxylamino and nitroso derivative of Everninomicin, an antibiotic (Fig. 12). An indication for direct oxygen transfer from the iron(V) oxo to the nitroso compound was derived from labelling experiments which showed that the oxygen in the nitro group was predominantly derived from H_2O_2.

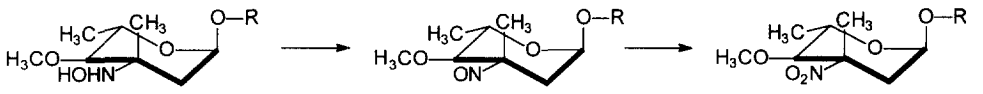


Fig. 12. *N*-oxidation catalyzed by HRP

N-oxidation of anilines to nitrobenzenes is reported to be catalyzed by a couple of non-heme bacterial peroxidases[63-65]. Whereas the chloroperoxidases from *Pseudomonas pyrrocinia* and *Serratia marcescens* can catalyze the oxidation of 4-chloro-aniline and 3-(2-amino-3-chloro-phenyl)-pyrrole, the bromoperoxidase from *Pseudomonas putida* catalyzes only the oxidation of aniline to nitrobenzene.

Epoxidation

Optically active epoxides are very useful chiral synthons as they can undergo facile stereospecific ring-opening to form bifunctional compounds[66]. They are important as key intermediates in the production of bioactive chiral compounds or as end products with biological activity. For example chiral epoxides are used for the production of β-blockers, the HIV protease inhibitor Crixivan[67] or calcium antagonists[68] like the drug Diltiazem (Fig. 13).

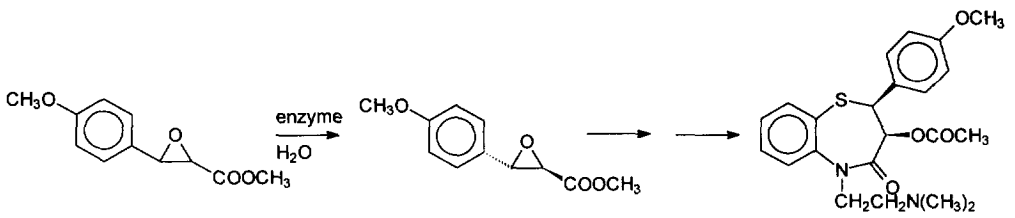


Fig. 13. Asymmetric synthesis of Diltiazem

Because of this important role for epoxides in organic synthesis much research is focused on the development of catalysts which can perform highly enantioselective epoxidation of unfunctionalized olefins[69]. Such catalysts can be divided into three classes: synthetic catalysts[70,71], enzymes[72] and catalytic antibodies[73]. However, the use of catalytic antibodies for enantioselective epoxidations is still in its infancy.

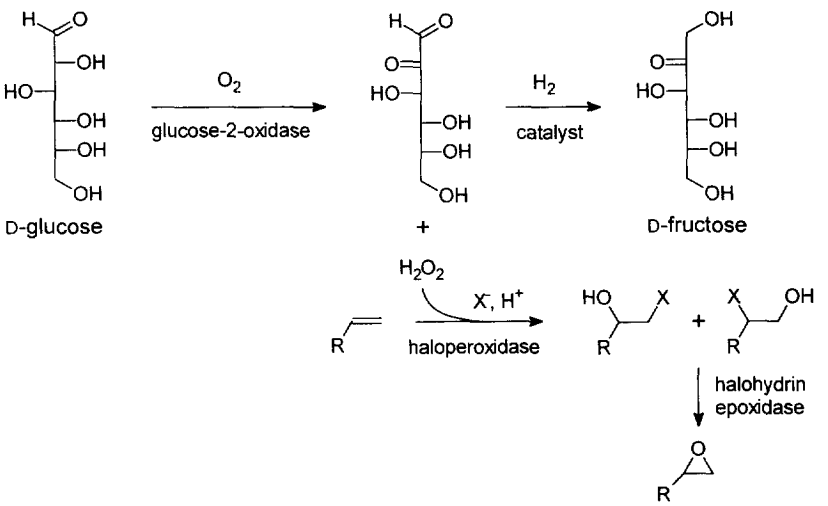


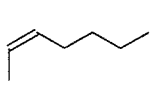
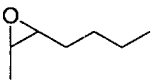
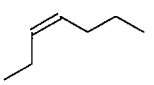
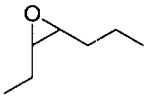
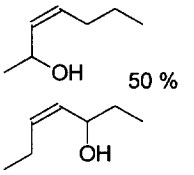
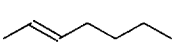
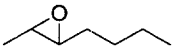
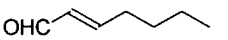
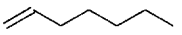
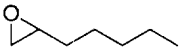
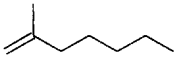
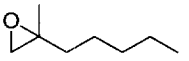
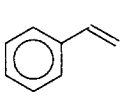
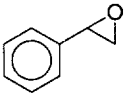
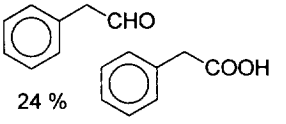
Fig. 14. Process for production of epoxides by haloperoxidase-catalyzed halohydrin formation

There are several approaches for the use of peroxidases for epoxidation. One approach is indirect epoxidation: *i.e.* haloperoxidase catalyzed formation of a halohydrin which can be converted chemically or with a halohydrin epoxidase to a (chiral) epoxide. A process for the production of propylene oxide[74] was developed using this approach with *in situ* generation of H_2O_2 by glucose oxidase (Fig. 14). However the process has not been commercialized. An important drawback of the halohydrin method is that the produced halohydrins are racemic and the epoxides produced with this method have a low enantiomeric excess.

Another example of the indirect oxidation of alkenes to epoxides catalyzed by peroxidases is the glutathione or 4-methylphenol dependent cooxidation of styrene to styrene oxide catalyzed by HRP. This reaction

yields racemic styrene oxide and benzaldehyde[75,76]. The last example of indirect oxidation is the epoxidation of alkenes by metal-free peroxidases yielding racemic epoxides[77]. The reaction can only be performed in acetate or propionate buffer and is believed to proceed via an enzymatically generated peracid, analogous to the lipase catalyzed oxidation of carboxylic acids. The peracid subsequently oxidizes the alkenes to racemic epoxides.

Table 4. CPO-catalyzed epoxidation of olefins[84,87]

Olefin	Product yield, *ee*	Byproducts yield	Turnover number
	 yield 100 %, *ee* 95 %		2100
	 yield 40 %, *ee* 95 %	 50 %	840
	 yield 3 %	 35 %	63
	 yield 2 %, *ee* 10 %		34
	 yield 23 %, *ee* 95 %		1700
	 yield 40 %, *ee* 49 %	 24 % 5 %	840

A more powerful method for producing epoxides using peroxidases is the direct CPO-catalyzed oxidation of alkenes by H_2O_2[78-81]. CPO catalyzes the epoxidation of *trans*-[1-^{2}H]styrene with retention of stereochemistry[80]. It was demonstrated that all oxygen in styrene oxide is derived from hydrogen peroxide, which implies that the reaction is a true oxygenation reaction. Phenylacetaldehyde is produced as a byproduct in quite large yields (50%). The same effect is observed with the epoxidation of butadiene, where crotonaldehyde is produced in 25% yield[81].

The enantioselective CPO-catalyzed epoxidation of alkenes was discovered just recently[82,83], although the CPO catalyzed epoxidation of olefins had been known for nearly a decade. Direct epoxidation (Table 4) catalyzed by CPO proceeds well, with high yield and excellent enantiomeric excess, for short-chain alkenes (up to C_9) with the double bond close to the chain terminus[83-85]. In contrast, epoxidation of *trans*-alkenes gave very low yields of epoxide. The major reaction was allylic oxidation, accompanied by further oxidation of the allylic alcohols to the corresponding aldehydes. Similarly, allylic hydroxylation was observed with alkenes in which the double bond was far removed from the chain terminus (*cis*-3-alkenes). Terminal alkenes gave rise to heme alkylation and subsequent protein deactivation[86]. Consequently, unbranched long-chain aliphatic 1-alkenes are poor substrates for epoxidation. The low turnover numbers for terminal alkenes can be increased by using short-chain prochiral terminal dienes or branched 1-alkenes[84,87]. Styrenes yielded a mixture of the epoxides and the isomeric phenylacetaldehydes and the obtained enantiomeric excess was moderate.

Microperoxidase-11, an undecapeptide containing a heme unit, can also catalyze the direct oxidation of olefins to epoxides[88]. However, aldehydes were obtained as side-products (71% for styrene and 33% for *cis*-stilbene), turnover numbers were low (up to 4 for styrene oxide) and enantiomeric excesses were not reported.

Another peroxidase which can catalyze the epoxidation of styrenes is cytochrome *c* peroxidase[47]. However, again turnover numbers were low (<1 during 20 min. reaction time), enantiomeric excesses were moderate (up to 32% *ee*) and for *cis*-styrenes partial rearrangement to the aldehyde or ketone occurred. Moreover, in the case of *cis*-β-methyl styrene the *trans*-epoxide was formed as a byproduct, possibly via a protein peroxyl radical mechanism.

Native HRP generally does not perform epoxidations, with the exception of *trans*-β-methyl styrene which was epoxidized in a low enantiomeric excess (*ee* = 6%)[50]. Various mutants of HRP (F41L, F41T, F41A, H42A, H42V) in contrast, catalyze the enantioselective epoxidation of styrene derivatives[50,51]. Mutants with increased accessibility to the active site were shown to catalyze the oxidation of styrene, *cis*-β-methyl styrene and *trans*-β-methyl styrene oxidation. However, a large amount of benzaldehyde and the rearranged aldehyde or ketone was obtained. Moreover, in the case of *cis*-methyl styrene the *trans*-epoxide was also obtained in low enantiomeric excess. The oxidation appears to involve more than a direct oxygen transfer from the iron(V) oxospecies to the olefin as labelling experiments showed that a substantial part of the oxygen in the product is derived from molecular oxygen. Only oxidation of *cis*-β-methyl styrene, catalyzed by the F41T mutant of HRP, gave complete enantioselecivity to the (1*S*,2*R*) enantiomer and in this case all the oxygen in the product was derived from hydrogen peroxide. The synthetic utility of these reactions is questionable, as the rates and enantiomeric excesses are generally low and substantial amounts of byproducts are obtained. Moreover, such mutants are not yet commercially available.

Finally, we want to mention epoxidations catalyzed by a soybean peroxygenase, which is related to peroxidases (Fig. 15). Oxidation of *cis* mono- and polyunsaturated fatty acids with cumene hydroperoxide or

long chain unsaturated alkyl hydroperoxides such as 13-hydroperoxylinoleic acid in the presence of soybean peroxygenase gave the corresponding epoxides in moderate enantiomeric excess[89].

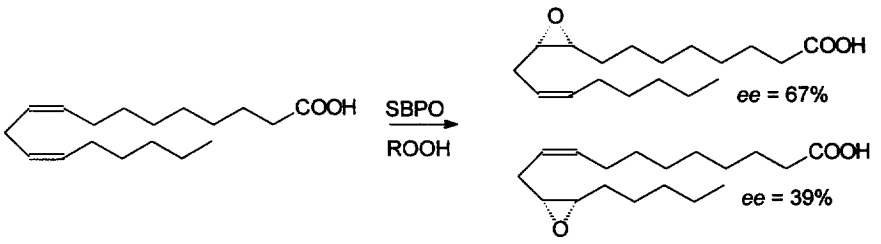


Fig. 15. Oxidation of alkenes by soybean peroxygenase (SBPO)

C-H bond oxidation

Benzylic/allylic hydroxylation. Selective hydroxylation of hydrocarbons is difficult to perform chemically. One of the few examples of selective benzylic hydroxylation mediated by a chemical chiral catalyst is shown in Fig. 16 in which a chiral manganese salen complex is used as the catalyst[90].

Nuclear hydroxylation of aromatic compounds is a common peroxidase reaction which generally yields polymerized products. However, selective hydroxylation of benzylic or allylic C-H bonds is rarely observed with peroxidases, although catalysis of this reaction by heme-containing mono-oxygenases is quite common[91].

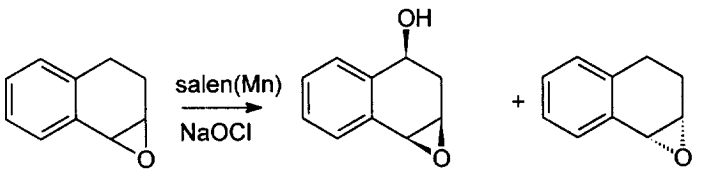
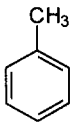
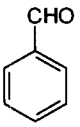
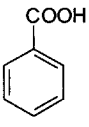
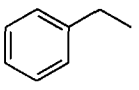
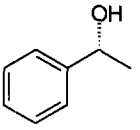
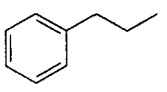
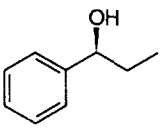
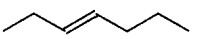
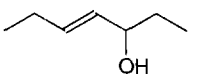
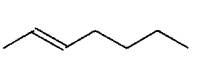
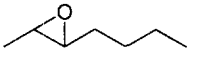
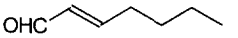


Fig. 16. Benzylic hydroxylation catalyzed by a chiral salen(Mn) complex

The only peroxidase known so far to give selective hydrocarbon hydroxylation is CPO (Table 5). The first study describing CPO-mediated hydrocarbon hydroxylation concerned the oxidation of cyclohexene to cyclohex-2-en-1-ol[78]. Recently two more thorough studies of CPO-catalyzed benzylic and allylic hydroxylation of hydrocarbons appeared[84,92]. The oxygen atom in benzylic alcohol was shown to be derived from H_2O_2 consistent with a mechanism involving direct oxygen transfer. *O*-Dealkylation was a major side reaction when *p*-methyl anisole was oxidized indicating that the reaction probably proceeds via initial one-electron transfer from the aromatic ring. Although in the study of Miller *et al.*[92] benzylic hydroxylation is a very slow reaction yielding total turnover numbers between 0.2 and 5, Zaks and Dodds[84] reported considerably higher turnover numbers (Table 5). Remarkable is the reversion in stereochemical preference of the obtained chiral benzylalcohol when the alkyl chain is enlarged from ethyl to propyl. Inversion of stereochemistry was also

observed in CPO-catalyzed epoxidations and it would be interesting to perform docking experiments using the recently resolved crystal structure of CPO[93] to find an explanation for these results.

Table 5. Benzylic hydroxylation catalyzed by CPO[84]

Substrate	yield product / byproduct		turnover number [a]
	 85%	 15%	2100 (1800+300)
	 20%, *ee* 97%		420
	 20%, *ee* 88%		420
	 6.0%		126
	 3.0%	 35%	735

a. Turnover number = mole of product produced per mole of enzyme used.

Alcohol oxidation. Oxidation of alcohols to aldehydes is quite common with dehydrogenases, monoxygenases and oxidases. Peroxidases, on the other hand, are generally restricted to oxidation of phenols to quinones via free radical intermediates (classical peroxidase reaction). Again chloroperoxidase from *Caldariomyces fumago* is the exception to the general rule and can catalyze the oxidation of primary alcohols to aldehydes[94,95]. CPO has a preference for alcohols which contain an allylic, propargylic or benzylic group. Although Geigert *et al.*[95] reported that the reaction is selective and no oxidation to the corresponding acid occurs, we have shown[96] that reactive aldehydes like 5-hydroxymethyl-furfural are oxidized to the

corresponding acid (Fig. 17). Oxidation of aldehyde to acid proceeds via direct oxygen transfer as complete incorporation of oxygen from $H_2{}^{18}O_2$ is observed.

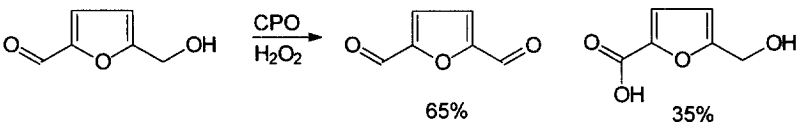


Fig. 17. Oxidation of 5-hydroxymethyl-furfural catalyzed by CPO[96]

Indole oxidation. Substituted oxindoles are interesting compounds due to their biological properties. 5-Chloro-oxindole, for example, is an intermediate in the synthesis of Tenidap (1-carbamoyl-5-chloro-3-[hydroxy(2-thienyl)methylene]indole-2-(3H)-one, Fig. 18), an anti-inflammatory drug[97]. Direct oxidation of indole to 2-oxindole is difficult, as oxidation generally occurs at the more electron rich 3-position[98], if this position is not substituted, yielding indoxyl, a precursor for indigo[99].

Chemical methods available for producing oxindole mostly consist of multi-step procedures[100-103]. Often harsh conditions are necessary or low yields or isomeric mixtures are obtained. Direct oxidation of indole to oxindole, however, is efficiently catalyzed by CPO[104,105]. Indoles with substituents at the 4-, 5- or 6-position yield the corresponding oxindoles in nearly quantitative yield (Fig. 19, Table 6). Labelling experiments showed that the oxygen in the product is derived from H_2O_2 consistent with a mechanism involving direct oxygen transfer from the iron(V) oxo species to the indole[105].

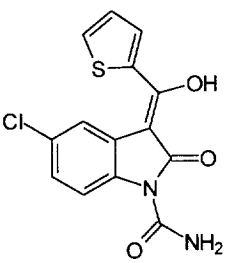


Fig. 18. Tenidap

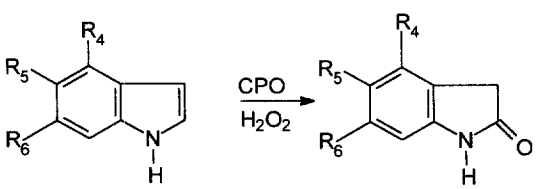


Fig. 19. Oxidation of substituted indoles by CPO

Table 6. Preparative scale synthesis of oxindole derivatives[105]

Indole derivative	CPO (kU) [a]	yield (%)	purity (%)
indole	1	96	96
7-aza-indole	2	97	99
4-Cl	6	70	76
5-Cl	2	99	99
5-Br	3	86	95
5-CH_3	6	92	94
5-OCH_3	6	93	95
6-Cl	2	96	99

a. Assay according to Morris and Hager[19]

3. MECHANISTIC ASPECTS

Peroxidases can be broadly divided into three categories based on the nature of the catalytic centre: heme peroxidases, vanadium haloperoxidases and other peroxidases.

Heme peroxidases

Catalytic center. The prosthetic group in this type of peroxidase[2,106-108] is a heme (generally ferri-protoporhyrin IX). In the native enzyme iron(III) is present in the high spin state. The iron is coordinated by the four nitrogen atoms of the heme. The 5th axial ligand of the iron is a histidine in most peroxidases. However, an exception is chloroperoxidase from *Caldariomyces fumago* which has a cysteine as the axial ligand. During catalysis the iron(III) species is oxidized by peroxide to a (formally) iron(V) oxo species, compound I. This reaction has generally been discussed in terms of a push/pull mechanism[109,110] as depicted in Fig. 20.

A basic amino acid residue (e.g. histidine) abstracts a proton from the hydroperoxide whilst stabilization of the developing negative charge on the peroxide by a positively charged amino acid, e.g. arginine, exerting a pull effect, and electron donation by the axial ligand at the proximal position - the push effect - would assist the heterolytic cleavage of the oxygen-oxygen bond. This attractive picture is not supported by recent experimental work, however. Mutation of the arginine (Arg 48) in cytochrome *c* peroxidase only marginally affected the rate of compound I formation[111], besides, X-ray analysis of a cytochrome *c* peroxidase oxygen complex revealed[112] that the arginine residue (Arg 48) is too far removed from the peroxide for any stabilizing interaction. It has also been shown that the axial ligand at the proximal position exerts only a small effect on the activation of the peroxide[113].

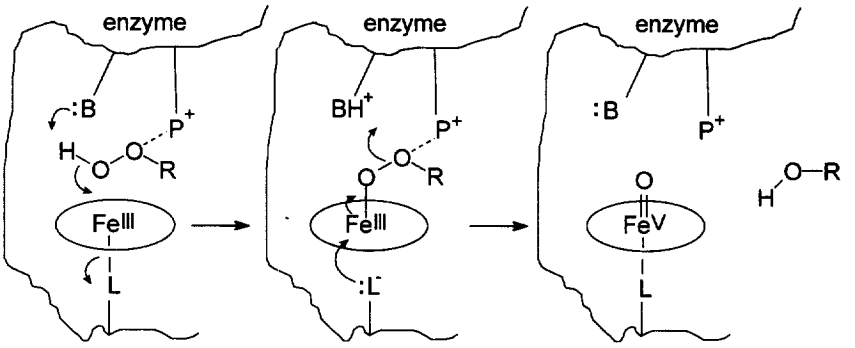


Fig. 20. Presumed mechanism for heterolytic cleavage of hydroperoxides catalyzed by peroxidases

Various combinations of amino acid residues are present in the active sites of different heme enzymes. For example in horseradish peroxidase and cytochrome *c* peroxidase the distal base and the proximal ligand are histidines. A hydrogen bridge between an aspartate and the proximal histidine provides an imidazolate which is a better electron donor. In CPO the distal base is presumed to be a glutamate whereas the proximal ligand is a thiolate which is a stronger electron donor than an imidazolate[93]. The heme iron redox potential is believed to be dependent on the stabilization of iron(V) oxo species (compound I). For example in cytochrome *c* peroxidase the binding of the aspartate to the proximal histidine increases the negative charge of the proximal ligand[114], thus stabilizing higher oxidation states of the heme iron and increasing the stability of compound I. Thus, the redox potential of cytochrome *c* peroxidase is decreased. In lignin peroxidase a strong hydrogen bonding network of a serine to an aspartate causes a movement of the proximal histidine containing helix, resulting in a weaker ligation of the proximal histidine to the heme iron[115]. Consequently, the heme in lignin peroxidase is more electron deficient than in cytochrome *c* peroxidase, which is believed to cause the higher oxidation potential of lignin peroxidase. In CPO the thiolate ligand is surrounded by a positive electrostatic environment which decreases the stabilization of compound I and increases the oxidation potential[93,115]. In most peroxidases, compound I is present as an iron(IV) oxo porphyrin radical cation species. In some peroxidases like cytochrome *c* peroxidase, lactoperoxidase and thyroid peroxidase the second oxidation equivalent of compound I is further delocalized to the protein moiety yielding an iron(IV) oxo protein radical cation species[117-119].

Compound I can be directly reduced to native enzyme by oxygen transfer (oxygenation, Fig. 21.3) or in two one-electron transfer steps (classical peroxidase reaction, Fig. 21.2). Oxygen transfer can proceed via a concerted two-electron transfer reaction or a two-step mechanism (oxygen rebound, *vide infra*). Electron transfer generally takes place at the heme edge or via protein radicals. Sometimes redox enzymes make use of a mediator to transfer electrons to a substrate which is too bulky to enter the active site. Veratryl alcohol, for example, mediates the oxidative degradation of lignin by lignin peroxidase[120,121]. Another example is

manganese (Mn^{2+}) which is oxidized by manganese dependent lignin peroxidase to Mn^{3+} which subsequently can oxidize phenolic compounds outside the enzyme[122,123]. Oxidation of cytochrome *c* by cytochrome *c* peroxidase takes place at the peripheral protein moiety via an electron transfer mechanism through the amino acid residues of the enzyme[124].

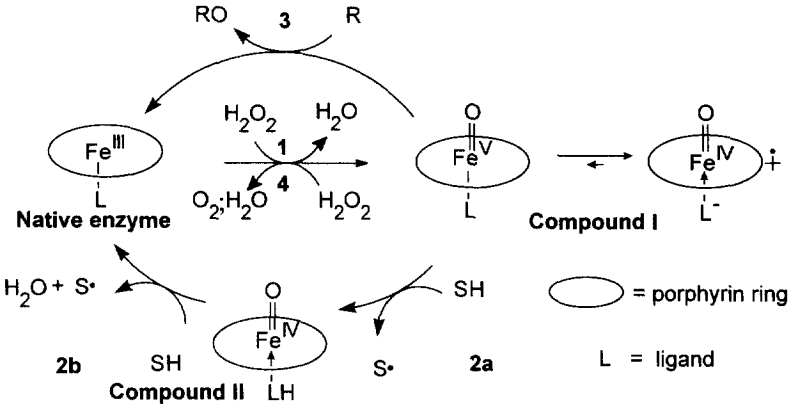


Fig. 21. Reaction cycle of peroxidases

Oxygen transfer from oxidized enzyme to substrate is only possible when the heme iron is accessible for the substrates. Ortiz de Montellano *et al.* performed several studies to investigate the accessibility of the active site of a range of peroxidases. The authors used phenyl and alkyl hydrazines and azide to react oxidatively with the peroxidase and studied the formed adducts. Reaction of HRP with these derivatives yields heme adducts at the δ-meso carbon[125-127]. Other peroxidases like *Coprinus* peroxidase[128], lignin peroxidase[129] and manganese peroxidase[130] yield similar results with phenyl hydrazine or azide, azide and ethyl hydrazine or azide, respectively. However, addition of phenyl or alkyl hydrazine to more accessible enzymes like catalase and chloroperoxidase results in covalent binding of the alkyl or aryl moiety to the iron and/or nitrogens of the porphyrin[131,132]. With CPO phenylhydrazine yields a phenyl-iron adduct and azide yields a δ-meso azido heme indicating that CPO can catalyze oxygen transfer reactions as well as the classical peroxidase reaction. Addition of phenyl hydrazine to cytochrome *c* peroxidase similarly yields a phenyl-iron adduct, although the crystal structure of the enzyme shows that the active site is buried in the protein[114]. Probably some small distortion of the active site affords a more open structure which can accommodate a phenyl group, which also may account for the sulfoxidation and epoxidation activity of cytochrome *c* peroxidase.

Crystal structures of other peroxidases like lignin peroxidase[115,133], *Coprinus* peroxidase[134] and manganese peroxidase[135] reveal that the active site is buried in the molecule, whereas CPO has a more open active site structure[93] allowing access to the heme iron for substrates with a limited size.

Mechanism of oxygen transfer. There are various mechanisms possible to account for direct oxygen transfer from the oxidized heme peroxidase to organic substrates. The oxygenations can be divided into oxygen transfer to alkenes, oxygen transfer to nitrogen or sulfur, C-H bond hydroxylation and indole oxidation.

Oxygen transfer to alkenes. Possible mechanisms for alkene oxidation are the shown in Fig. 22. Please note that the various intermediates are intraconvertible via electron transfer processes.

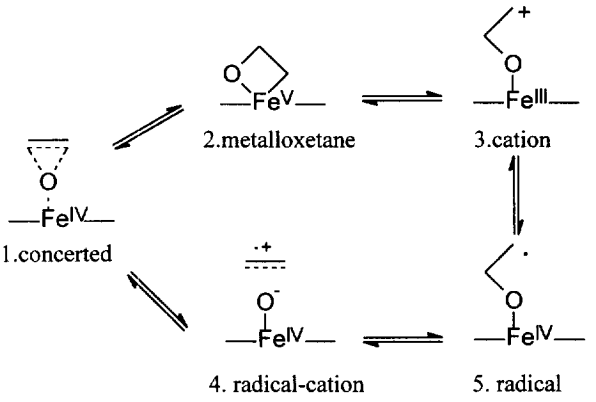


Fig. 22. Possible intermediates in oxygen transfer to alkenes

1. Concerted mechanism: (Fig. 22.1). the oxidation proceeds in one, concerted, step.
2. Oxygen transfer via a radical cation. (Fig. 22.4). In this mechanism initial one-electron transfer is followed by oxygen transfer from the iron(IV) oxo species to the radical cation intermediate (oxygen rebound). This intermediate has been trapped for a synthetic iron(IV) oxo porphyrin radical cation catalyzed epoxidation of styrene[136].
3. The formation of a cationic (Fig. 22.3) or a radical (Fig. 22.5) intermediate via direct electrophilic attack of the iron(V) oxo-species on the electron rich substrate or via further reaction of the radical cation. If the intermediate is a cation and the carbon adjacent to the cation has a hydrogen-atom, an NIH-shift may occur in which a hydride is transferred from the neighbouring carbon to the cationic carbon (Fig. 23). This may account for the rearrangement products obtained when styrene derivatives or butadiene are oxidized by chloroperoxidase[80,81].
4. Oxygen transfer via a metalloxetane intermediate (Fig. 22.2). This intermediate has been proposed to be involved in biomimetic alkene epoxidations[137]. However, a metalloxetane intermediate is unlikely because of steric restrictions[138].

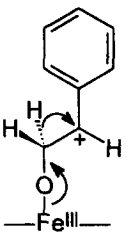


Fig. 23. Rearrangement to 2-phenyl-acetaldehyde

Oxygen transfer to sulfur and nitrogen. Oxidation at sulfur or nitrogen may proceed concerted, via a radical cation or via electrophilic attack of the iron(V) oxo species on the electron rich reactant analogous to the mechanisms described for alkene oxidation (1, 2 and 3 in Fig. 22). Evidence for a radical cation intermediate in sulfoxidation reactions has been obtained for lactoperoxidase[34] and the peroxidase related soybean peroxygenase[53]. There is also support that this mechanism occurs with horseradish peroxidase[139,140]. However, for chloroperoxidase the mechanism is less clear and the sulfoxidation might proceed either via a concerted mechanism or via two very fast subsequent one-electron transfer processes.

Table 7. Oxygen incorporation in methyl phenyl sulfide

Enzyme	$H_2{}^{18}O_2$ incorporation (%)
CPO[34]	99
HRP[34,*a*]	90
LPO[34,*b*]	85
MP-11[141]	100

a. $H_2{}^{18}O$ HRP yields 10% of oxygen incorporation from water for *p*-OCH_3 -phenyl methyl sulfide.
b. $^{18}O_2$: LPO yields 22% of oxygen incorporation from molecular oxygen for phenyl methyl sulfide.

In Table 7 the source of the oxygen in the sulfoxide is depicted for several peroxidases. Although for all peroxidases shown, the greater part of the oxygen is derived from hydrogen peroxide, indicating that the oxidation is an oxygen transfer reaction, horseradish peroxidase and lactoperoxidase also give oxygen incorporation from another source. Oxygen transfer from water to *p*-methoxy-phenyl methyl sulfide in the case of horseradish peroxidase (10% for *p*-methoxy-phenyl methyl sulfide, Table 7) might either be due to reaction of a sulfur dication with water or to exchange of the oxygen of the iron(IV) oxo species (compound II) with water[35,142]. A third possible explanation for oxygen transfer from water to the sulfide is depicted in Fig. 24. The intermediate sulfide radical cation reacts with water in the active site. Subsequently the hydroxy-sulfide

radical is further oxidized by compound II to yield the sulfoxide. The incorporation of oxygen derived from molecular oxygen in phenyl methyl sulfide for lactoperoxidase might be caused by formation of a protein peroxyl radical during catalysis[34].

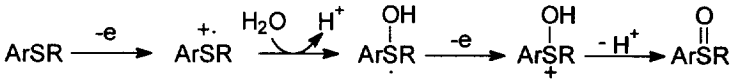


Fig. 24. Possible mechanism for oxygen transfer from water to sulfide

We note that hydroxylamine oxidation catalyzed by CPO and HRP might very well proceed via the classical peroxidase reaction (oxidative dehydrogenation) instead of via an oxygen transfer mechanism. There is no evidence for direct oxygen transfer from the enzymes to the hydroxylamine, contrary to chloroperoxidase-catalyzed oxidation of amines or horseradish peroxidase catalyzed oxidation of nitroso-compounds.

C-H bond hydroxylation. The mechanism of C-H bond hydroxylation is ambiguous, the reaction may proceed via a concerted mechanism or, alternatively, via hydrogen abstraction followed by oxygen rebound (Fig. 25). Hydrogen abstraction step may occur directly or via an intermediate carbocation (Fig. 27).

1. concerted **2. hydrogen abstraction**

Fig. 25. Possible mechanisms for C-H bond oxidation

1. Concerted mechanism (Fig. 25.1).

   Results obtained from experiments with a radical clock (Fig. 26) support a concerted mechanism for chloroperoxidase-catalyzed benzylic hydroxylation[84]. However, one should keep in mind that the active site of chloroperoxidase has a limited size and the rearrangement of the radical clock might be severely hindered by the restriction in the active site, thus a hydrogen abstraction mechanism cannot be completely ruled out. Deuterium labelling experiments indicate that cleavage of the C-H bond occurs in the rate limiting step[92].

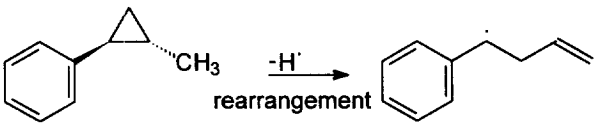


Fig. 26. Rearrangement of radical clock if hydroxylation proceeds non-concerted

2. Hydrogen abstraction (Fig. 25.2).

   In this mechanism hydrogen abstraction is followed by hydroxyl transfer from the enzyme to the substrate. The hydrogen abstraction may proceed either directly or in two steps: first electron transfer followed by proton elimination (Fig. 27).

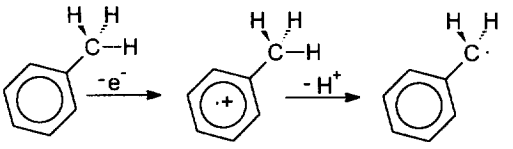


Fig. 27. Electron transfer followed by proton abstraction

The oxidation of alcohols to the corresponding aldehydes is generally restricted to allyl and benzyl alcohols; its mechanism is ambiguous. A dehydrogenation (abstraction of two hydrogen atoms) would yield the alcohol directly. Alternatively, hydrogen abstraction from the carbon atom followed by oxygen rebound would give rise to a *gem*-diol which converts to the aldehyde.

Indole oxidation. The oxidation of indole to oxindole is a special case of C-H bond oxidation as the hydroxylated carbon is part of an aromatic system. This is also the case in phenol hydroxylation, a classical peroxidase reaction. A concerted mechanism for indole oxidation is questionable as an epoxide would be expected as intermediate instead of direct C-H bond hydroxylation. The formation of this epoxide intermediate is highly unlikely: the oxidation of alkenes proceeds much slower than the oxidation of indole. Direct electrophilic attack of compound I on indole is not expected to occur at the 2-position as the 3-position is the most electron rich one. However, the active site pocket might provide an environment in which the 2-position becomes the favourite position for electrophilic attack. A metalloxetane intermediate is unlikely due to the earlier mentioned steric constraints.

By analogy with reactions of peroxidases with other aromatic substrates we propose[105] a mechanism involving initial one-electron transfer to produce a radical cation intermediate. Subsequent oxygen rebound followed by electron redistribution and proton loss yield the hydroxylated indole which tautomerized to the more stable oxindole (Fig. 28).

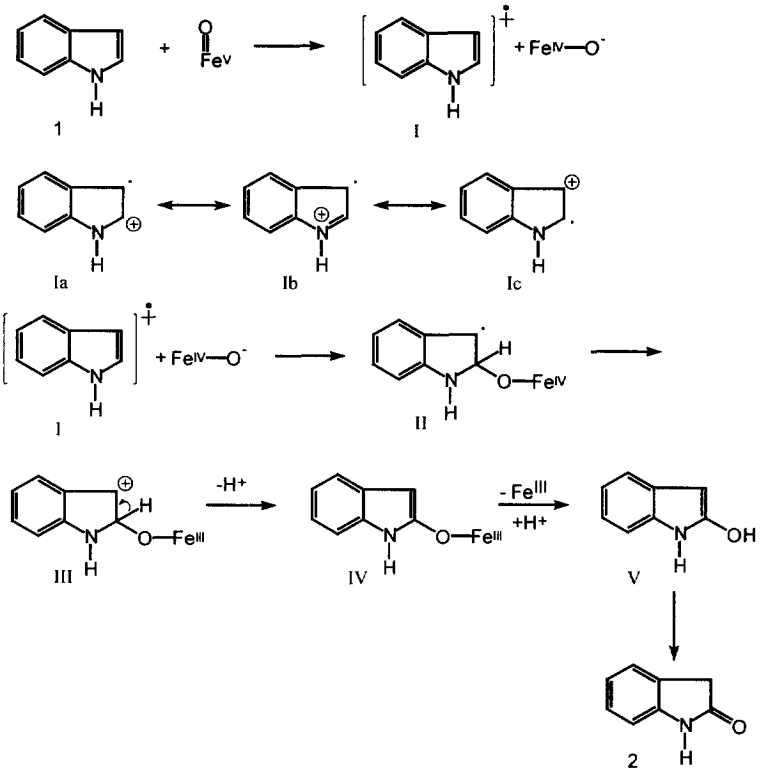


Fig. 28. Proposed mechanism for oxidation of indole to oxindole by CPO

Deactivation of heme peroxidases. Heme peroxidases are prone to deactivation *via* three different pathways. In this review we will discuss oxidation of the porphyrin ring by the oxidant (irreversible), reaction of enzyme with suicide inhibitors (irreversible) and compound III formation (reversible) as deactivation pathways for heme peroxidases.

Oxidation of the porphyrin ring by the oxidant. Heme enzymes are susceptible to oxidative destruction of the porphyrin ring. For example the heme of cytochrome P-450 is rapidly destroyed by oxidants like hydrogen peroxide, alkyl hydroperoxides and iodosobenzene[143]. The oxidative destruction of HRP by hydrogen peroxide or *m*-chloroperbenzoic acid yields an unstable verdohemoprotein called P-670[144,145]. Oxidation of heme by heme oxygenase yields biliverdin which involves α-meso-hydroxylation of the porphyrin ring and subsequent fragmentation to verdoheme[146]. The oxidation of the heme moiety may involve various different activated oxygen species, *e.g.* hydrogen peroxide, superoxide anion, hydroxyl radicals or singlet oxygen. All these activated oxygen species may be produced during the catalytic cycle of peroxidases and may damage the peroxidase irreversibly. Vanadium peroxidases are far more stable towards oxidative destruction than heme peroxidases as they do not contain a porphyrin ring[147].

Suicide deactivation. Peroxidases can be deactivated by reaction with so-called suicide inhibitors formed *in situ* during catalysis. Phenylhydrazine, alkyl hydrazines and sodium azide are often used to perform mechanistic and active site topology studies with peroxidases. Reaction of these compounds with peroxidase yields free radicals that form adducts with the peroxidase, either at the *meso*-heme-edges, the iron or the porphyrin nitrogens, which yields information about the active site topology of peroxidases (*vide supra*).

In contrast with the above mentioned inhibitors which are deliberately added to the reaction mixture deactivation can also be due to reactive intermediates formed during the reaction. An example is the heme alkylation by the epoxide product int the CPO-catalyzed epoxidation of 1-alkenes[86]. Similarly, resorcinol derivatives can deactivate lactoperoxidase and thyroid peroxidase during catalysis[148] probably due to the formation of reactive radicals which react with amino acid residues in these enzymes. Another example of a suicide inhibitor is phenol. During the oxidation of phenol HRP is deactivated. At low phenol concentrations this is due to accumulation of compound III (see next paragraph) and deactivation is reversible. At high phenol concentrations deactivation is irreversible and phenol concentration dependent[149]. This irreversible deactivation might well be due to the formation of phenoxy radicals which react with oxygen to form a reactive peroxy-radical species which destroys the enzyme[150].

Compound III formation. A third pathway for deactivation of heme peroxidases is the formation of a so-called Compound III intermediate. Compound III is often referred to as oxyperoxidase as it can be obtained by the addition of oxygen to the ferrous peroxidase[151-153]. Other means for obtaining compound III are adding excess hydrogen peroxide to either native enzyme or compound II[153,154] or adding superoxide anion to the native enzyme[155].

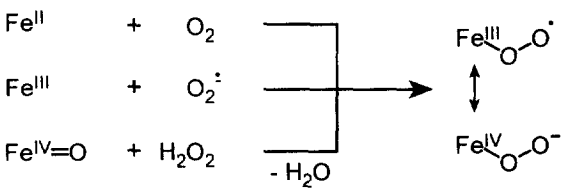


Fig. 29. Formation and mesomeric structures of compound III

Compound III has been represented in several isoelectronic structures, all of which have found some experimental support[156-159]. The closely related oxyhemoglobin has been characterized as a low-spin iron(III) superoxide species on the basis of its diamagnetism; the oxygen is bound to the iron atom with a bent, end-on geometry[160] (Fig. 29), as predicted by Pauling and Coryell[161]. The structure of compound III derived from a cytochrome *c* peroxidase mutant, which has been resolved by X-ray diffraction[112], shows a similar arrangement. The iron-oxygen bond distances are nearly equal in both cases (1.75 and 1.8 Å, respectively).

Accumulation of compound III during turnover leads to less enzyme available for catalysis and apparent enzyme deactivation, as compound III is not involved in the peroxidase reaction cycle.

The stability of compound III depends on the peroxidase. For CPO it has been shown that compound III is very unstable and converts to ferric enzyme by autoxidation without detectable intermediates when no hydrogen peroxide is present[151]. Lignin peroxidase, in contrast, is stable for several days when excess hydrogen peroxide is removed[153,162]. It has been shown recently that radical cations can revert compound III of LiP to native (ferric) enzyme. Thus, the apparent deactivation due to compound III formation is reversible for lignin peroxidase[162,163]. A possible mechanism for the reaction cycle of compound III formation and conversion to native enzyme for lignin peroxidase is shown in Fig. 30. The crucial step for converting compound III to native enzyme is its one-electron oxidation by a radical cation to a (formally) iron(V) peroxo species which spontaneously loses oxygen. This intermediate is comparable to the intermediate which we have proposed for the catalase reaction catalyzed by CPO[164] and which is referred to in the literature[22] as a compound I.H_2O_2-species. Its electronic structure is not known but some plausible candidates are depicted in Fig. 30. It is pertinent to note that the iron(IV) superoxide species would yield triplet oxygen, whereas the iron peroxo complexes would be expected to decay to singlet oxygen. Unfortunately it is not known whether singlet or triplet oxygen is formed in these processes.

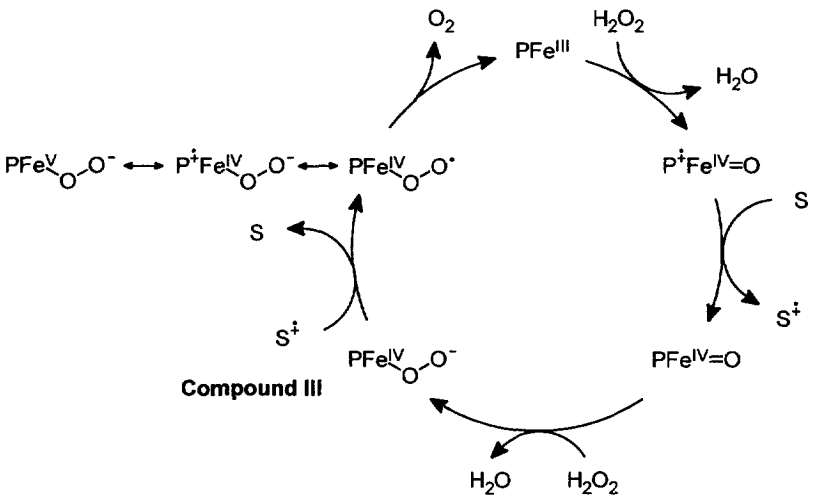


Fig. 30. Proposed mechanism for regeneration of native lignin peroxidase from compound III

Thus, when reductants are present which form stable radical cations, decay of LiP compound III proceeds via a reversible pathway in which hydrogen peroxide is decomposed to oxygen and water. However, with excess hydrogen peroxide and no reductant the decay of compound III of LiP and other peroxidases

partially proceeds irreversibly, due to the formation of reactive oxygen intermediates which destroy the enzyme[149,165-167]. In this case the decay of compound III to ferric peroxidase is believed to proceed via ferrous enzyme and compound II[167,168].

Vanadium haloperoxidases

Next to heme-containing peroxidases it has recently become clear that a second class of haloperoxidases exists which contains vanadate as the prosthetic group. These vanadium peroxidases are isolated mainly from the marine environment, *e.g.* a bromoperoxidase from brown seaweed *Ascophyllum nodosum*[169,170], but also from other sources like the terrestrial fungus *Curvularia inaequalis* from which a chloroperoxidase has been isolated[171]. These vanadium haloperoxidases show considerable stability towards organic cosolvents[172-174]. Furthermore, in contrast to heme peroxidases, they are very stable under oxidizing conditions[147]. The oxidation state of vanadate does not change during catalysis. Reaction of hydrogen peroxide with the vanadium(V) probably affords an η-peroxovanadium(V) complex, comparable to known biomimetic η-peroxovanadium(V)-complexes[156]. This complex subsequently oxidizes the halogen to hypohalite[175,176].

Recently the crystal structure of a vanadium chloroperoxidase from *Curvularia inaequalis* azide complex has been resolved[177] and it was shown that the prosthetic group consists of hydrogen vanadate(V) in a trigonal bipyramidal coordination. The vanadium is coordinated to three non-protein oxygens, one nitrogen from a histidine and one nitrogen from the bound azide. The protein fold is highly α-helical and the helices are packed together in a compact structure accounting for the high stability of the haloperoxidase. However, accessibility to the active site is limited to small molecules like halide, thus limiting the synthetic utility of vanadium chloroperoxidase.

Other peroxidases

Several bacterial haloperoxidases which contain neither heme nor vanadium as the prosthetic group have been isolated recently[20,63,65,178-180]. They catalyze the bromination, but not the chlorination of monochlorodimedone. Furthermore some of these enzymes catalyze the chlorination of indole and the oxidation of anilines to the nitro-compounds[63-65,180]. Selective chlorination of indole to 7-chloro-indole was demonstrated for the chloroperoxidase from *Pseudomonas pyrrocinia*[20].Only the bromoperoxidase from *Pseudomonas putida* has been shown to give substrate specificity for aniline in the oxidation of anilines to the nitro-compounds[180].

The non-heme, non-vanadium haloperoxidases exhibit their halogenating properties only when used in acetate or propionate buffer. Furthermore, for the bromoperoxidase from *Streptomyces aureofaciens* the crystal structure shows that the enzyme contains the same catalytic triad (Asp-His-Ser) as the serine-proteases and lipases[181]. It is highly likely, therefore, that the catalytic mechanism involves perhydrolysis of an acylenzyme

intermediate to peracetic acid which reacts outside the active center, analogous to the mechanism observed with lipases[182] (see Fig. 31). Similarly, oxidations mediated by other bacterial non-heme, non-vanadium haloperoxidases may also involve peracid intermediates.

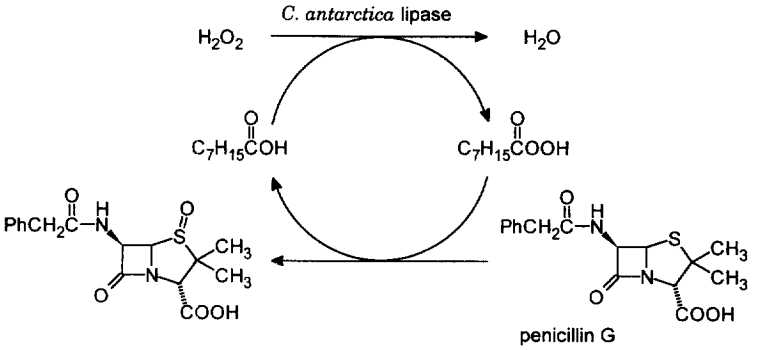


Fig. 31. Lipase mediated oxidation of penicillin G to its sulfoxide

Glutathione peroxidase contains a selenocysteine as the prosthetic group[183]. It catalyzes the oxidative dimerization of glutathione using a hydroperoxide as the primary oxidant. The enzyme has a low specificity towards the hydroperoxide, however the specificity for glutathione is very high and only low activities were observed for thiols other than glutathione. The oxidation presumably proceeds via a selenic acid derivative as depicted in Fig. 32.

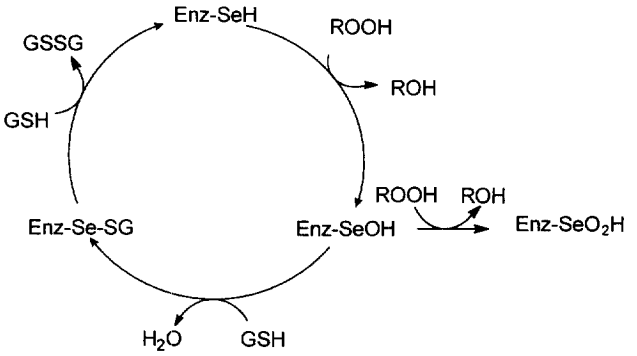


Fig. 32. Reaction cycle of glutathione peroxidase

Recently a novel non-heme, metal containing peroxidase has been discovered which is involved in lignin degradation[184]. The precise structure of the active site and the reaction mechanism of this peroxidase is not yet clear and further research is needed to determine to which class of peroxidases this enzyme belongs. The enzyme showed classical peroxidase activity and could degrade wheat straw in the presence of hydrogen peroxide.

4. COMMERCIAL APPLICATIONS AND CONCLUSIONS

Peroxidases have potential commercial applications in many different areas. The most developed field for their commercial application is in analytical diagnostics, for example in biosensors and immunoassays[185-187]. Moreover peroxidases are being extensively studied as bleaching inducer in, for example, the detergent and pulp industries[188-191], in anti-microbial applications[192-194], for removing aromatics from waste streams[195,196] and as a catalyst for oxidative coupling[197], for example as a catalyst for dough preparation[198] (Fig. 33). Recently, attention is focused on the use of peroxidases as catalysts for selective oxygenations (this review) and as mild polymerization catalysts.

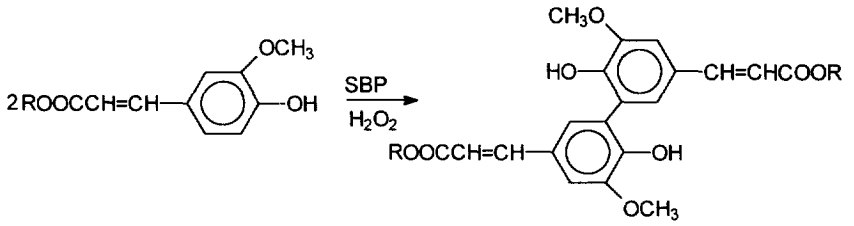


Fig. 33. Oxidative coupling of a substituted ferulic acid yielding a carbohydrate gel which can retain water well, R=arabinoxylan. Process developed by Quest Int. Naarden, The Netherlands

Although peroxidases have potential for a variety of commercial applications and techniques are available to produce them on a large scale[199-201] commercial processes using peroxidases as catalysts in organic synthesis are still limited. Limited commercial availability and stability and the relatively low productivities (space-time yield) are important features which encumber the commercial application of peroxidases. Moreover, heme peroxidases show a low stability towards the oxidant. This problem can be circumvented by using haloperoxidases like vanadium chloroperoxidase which are more stable. However, the only reaction known to be catalyzed by these enzymes is hypohalite production. Other means to avoid destruction of heme peroxidases during catalytic turnover are enzyme modification[202] or process considerations like the mode of oxidant addition[74,203] or the use of cosolvents which may prevent suicide deactivation[164]. *tert*-Butyl alcohol is known to prevent suicide deactivation for the CPO catalyzed oxidation of indole (Fig. 34)[164].

Summarizing we conclude that peroxidases have potential as catalysts for selective oxygen transfer reactions, however the restricted active site of peroxidases limits their activity and therefore their synthetic utility as oxygen transfer catalysts. One positive exception is the chloroperoxidase from *Caldariomyces fumago* which has great potential as a selective oxygen transfer catalyst. However, instability of this heme peroxidase during catalytic turnover limits its synthetic utility, although recently a process for the oxidation of indole has been developed in which a catalyst life-time of over 850,000 total turnovers was achieved[204]. Furthermore, we expect that in the future new developments in the use of site-directed mutagenesis to design peroxidases with

increased accessibility to the metal center, developments to increase the operational stability of peroxidases and the isolation of new peroxidases with novel properties will extend their synthetic utility.

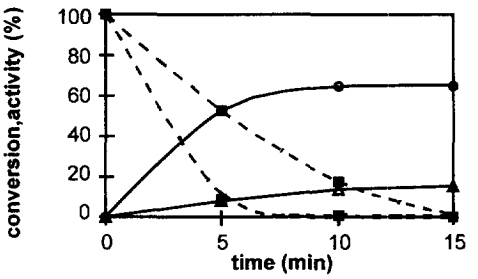


Fig. 34a. Indole oxidation in aqueous buffer; ○ conversion 300 U CPO/ 50 mL, Δ conversion 60 U CPO/ 50 mL, □ relative activity 300 U CPO/ 50 mL, ■ relative activity 60 U CPO/ 50 mL.

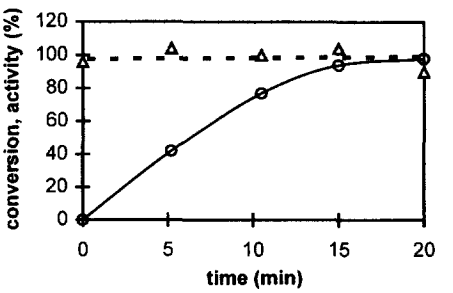


Fig. 34b. Indole oxidation in *tert*-butyl alcohol/ aqueous buffer pH 4 (30:70, v/v), ○ conversion, Δ relative activity

ACKNOWLEDGEMENT

The authors wish to thank Dr. U. Kragl (Research Center Jülich, Germany), Prof. Dr. H.E. Schoemaker and Dr. R. Wever (both of the University of Amsterdam, The Netherlands) for their helpful comments.

5. REFERENCES

1 Wichmann, R.; Wandrey, C.; Leuchtenberger, W.; Kula, M.R. and Bückmann, A.F. (Degussa; Gesellschaft für Biotechnologische Forschung) Ger. Offen. DE 2930087 [*Chem. Abstr.* **1981**, 94, 172901].

2. *Peroxidases in Chemistry and Biology*, Vol. I and II; Everse, J.; Everse; K.E. and Grisham, M.B. Eds.; CRC Press: Boca Raton, 1991.

3. *Plant Peroxidases in Biochemistry and Physiology*; Welinder, K.G.; Rasmussen, S.K.; Penel, C. and Greppin, H. Eds.; University of Geneva: Geneva, 1993.

4. Saunders, B.C.; Holmes Siedle, A.G. and Stark, B.P. *Peroxidase*; Buttersworths: Washington, 1964.

5. Dunford, H.B. *Adv. Inorg. Biochem.*, **1982**, 4, 41-68.

6. Hewson, W.D. and Hager, L.P. in *The Porphyrins* Vol. 7B; Dolphin, D. Ed.; Academic Press: New York, 1979; pp. 295-332.

7. Neidleman, S.L. and Geigert, J. *Biohalogenation, Principles, Basic roles and Applications*; Ellis Horwood Ltd.; Chicester: 1986.

8. Franssen, M.C.R. and van der Plas, H.C. *Adv. Appl. Microbiol.* **1992**, 37, 41-98.

9. Franssen, M.C.R. *Biocatalysis* **1994**, 10, 87-112.

10. Gallagher, P.H. *Biochem. J.* **1923**, 17, 515-529.

11. Fukunishi, K.; Kitada, K. and Naito, I. *Synthesis* **1991**, 237-238.

12. Zemel, H. and Quinn, J.F. (Allied Signal Inc.) PCT Int. Appl. WO 94 10327 [*Chem. Abstr.* **1994**, 121, 177877]; *Chem. Eng. News* **April 19, 1993**, 71, 36-37.

13. Dordick, J.S. *Tibtech.* **1992**, 10, 287-293.

14. Uyama, H.; Kurioka, H.; Sugihara J. and Kobayashi, S. *Bull. Chem. Soc. Jap.* **1996**, 69, 189-193.

15. Kobayashi, S.; Shoda, S. and Uyama, H. *Adv. Pol. Sci.* **1995**, 121, 1-30.
16. Meunier, B. in *Peroxidases in Chemistry and Biology*,Vol. II; Everse, J.; Everse, K.E. and Grisham, M.B. Eds.; CRC Press: Boca Raton, 1991; pp. 201-217.
17. Meunier, G. and Meunier, B. *J. Am Chem. Soc.* **1985**, 107, 2558-2560.
18. Shaw, P.D. and Hager, L.P. *J. Biol. Chem.* **1961**, 236, 1626-1630.
19. Morris, D.R. and Hager, L.P. *J. Biol. Chem.* **1966**, 241, 1763-1768.
20. Wiesner, W.; van Pée, K.-H. and Lingens, F. *FEBS Lett.* **1986**, 209, 321-324.
21. Arnao, M.B.; Acosta, M.; del Río, M.A.; Varón, R. and García-Cánovas, F. *Biochim. Biophys. Acta* **1990**, 1041, 43-47.
22. Sun, W.; Kadima, T.A.; Pickard, M.A. and Dunford, H.B. *Biochem. Cell. Biol.* **1994**, 72, 321-331.
23. Kanofsky, J.R. *J. Biol. Chem.* **1984**, 259, 5596-5600.
24. Everett, R.R. and Butler, A. *Inorg. Chem.* **1989**, 28, 393-395.
25. Hochman, A. in *Plant Peroxidases in Biochemistry and Physiology*; Welinder, K.G.; Rasmussen, S.K.; Penel, C. and Greppin, H. Eds.; University of Geneva: Geneva, 1993; pp. 103-112.
26. Barbachyn, M.R. and Johnson, C.R. in *Asymmetric Synthesis*; Morrison, J.D. Ed.; Academic Press: New York, 1988; p. 233.
27. Carreño, M.C. *Chem. Rev.* **1995**, 95, 1717-1760.
28. Andersen, K.K. *Tetrahedron Lett.* **1962**, 3, 93-95.
29. Noda, K.; Hosoya, N.; Irie, R.; Yamashita, Y. and Katsuki, T. *Tetrahedron*, **1990**, 50, 9609-9618.
30. Holland, H.L. *Organic Synthesis with Oxidative Enzymes*; VCH: New York, 1992; pp. 255-311.
31. Kagan, H.B. in *Catalytic Asymmetric Synthesis*, Ojima, I. Ed.; VCH: New York, 1993; pp. 203-226.
32. Dabrowicz, J.; Kielbasinski, P. and Mikolajczyk, M. in *The Chemistry of Sulphones and Sulphoxides;* Patai, S.; Rappoport, Z. and Stirling, C.J.M. Eds.; Wiley: Chicester, 1988; pp. 233-378.
33. Doerge, D.R. *Arch. Biochem. Biophys.* **1986**, 244, 678-685.
34. Doerge, D.R.; Cooray, N.M. and Brewster, M.E. *Biochem.* **1991**, 30, 8960-8964.
35. Kobayashi, S.; Nakano, M.; Goto, T.; Kimura, T. and Schaap, A.P. *Biochem. Biophys. Res. Commun.* **1986**, 135, 166-171.
36. Blée, E. and Durst, F. *Arch. Biochem. Biophys.* **1987**, 254, 43-52.
37. Kobayashi, S.; Nakano, M.; Kimura, T. and Schaap, A.P. *Biochem.* **1987**, 26, 5019-5022.
38. Colonna, S.; Gaggero, N.; Manfredi, A.; Casella, L.; Gullotti, M.; Carrea, G. and Pasta, P. *Biochem.* **1990**, 29, 10465-10468.
39. Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G. and Pasta, P. *Tetrahedron: Asymm.* **1992**, 3, 95-106.
40. van Deurzen, M.P.J.; Remkes, I.J.; van Rantwijk, F. and Sheldon, R.A. *J. Mol. Cat. A: Chemical* in press.
41. Pasta, P.; Carrea, G.; Colonna, S. and Gaggero, N. *Biochim. Biophys. Acta*, **1994**, 1209, 203-208.
42. Fu, H.; Kondo, H.; Ichikawa, Y.; Look, G.C. and Wong, C.-H. *J. Org. Chem.* **1992**, 57, 7265-7270.
43. Geigert, J.; DeWitt, S.K.; Neidelman, S.L.; Lee, G.; Dalietos, D.J. and Moreland, M. *Biochem. Biophys Res. Commun.* **1983**, 116 , 82-85.
44. Colonna, S.; Gaggero, N.; Carrea, G. and Pasta, P. *J. Chem. Soc., Chem. Commun.* **1992**, 357-358.
45. Harris, R.Z.; Newmyer, S.L. and Ortiz de Montellano, P.R. *J. Biol. Chem.* **1993**, 268, 1637-1645.
46. Casella, L.; Gullotti, M.G.; Ghezzi, R.; Poli, S.; Beringhelli, T.; Colonna, S. and Carrea, G. *Biochem.* **1992**, 31, 9451-9459.
47. Miller, V.P.; DePillis, G.D.; Ferrer, J.C.; Mauk, A.G. and Ortiz de Montellano, P.R. *J. Biol. Chem.* **1992**, 267, 8936-8942.
48. Colonna, S.; Gaggero, N.; Carrea, G. and Pasta, P. *Tetrahedron Lett.* **1994**, 35, 9103-9104.
49. Colonna, S.; Gaggero, N.; Richelmi, C.; Carrea, G. and Pasta, P. *Gaz. Chim. Ital.* **1995**, 125, 479-482.
50. Ozaki, S. and Ortiz de Montellano, P.R. *J. Am. Chem. Soc.* **1994**, 116, 4487-4488.
51. Newmyer, S.L. and Ortiz de Montellano, P.R. *J. Biol. Chem.* **1995**, 270, 19430-19438.
52. Ozaki S. and Ortiz de Montellano, P.R. *J. Am. Chem. Soc.* **1995**, 117, 7056-7064.
53. Blée, E. and Schuber, F. *Biochem.* **1989**, 28, 4962-4967.
54. Corbett, M.D.; Chipko, B.R.and Baden, D.G. *Biochem. J.* **1978**, 175, 353-360.

55. Corbett, M.D.; Baden, D.G. and Chipko, B.R. *Bioorg. Chem.* **1979**, 8, 91-95.
56. Corbett, M.D.; Chipko, B.R. and Batchelor, A.O. *Biochem J.* **1980**, 187, 893-903.
57. Corbett, M.D. and Corbett, B.R. *Biochem. Arch.* **1985**, 1, 115-120.
58. Doerge, D.R. and Corbett, M.D. *Chem. Res.Toxicol.* **1991**, 4, 556-560.
59. Ishimaru, A. and Yamazaki, I. *J. Biol. Chem.* **1977**, 252, 6118-6124.
60. Corbett, M.D. and Corbett, B.R. *J. Agric. Food Chem.* **1983**, 31, 1276-1282.
61. Holland, V.R. and Saunders, B.C. *Tetrahedron* **1968**, 24, 585-588.
62. Kalliney, S. and Zaks, A. *Tetrahedron Lett.* **1995**, 36, 4163-4166.
63. Itoh, N.; Morinaga, N. and Kouzai, T. *Biochem. Mol. Biol. Int.* **1993**, 29, 785-791.
64. Kirner, S. and van Pée, K.-H. *Angew. Chem., Int. Ed. Engl.* **1994**, 33, 352-353.
65. Burd, W.; Yourkevich, O.; Voskoboev, A.J. and van Pée, K.-H. *FEMS Microbiol. Lett.* **1995**, 129, 255-260.
66. Gorzynski Smith, J. *Synthesis*, **1984**, 8, 629-656.
67. Davies, I.W. and Reider, P.J. *Chem. Ind.* **1996**, 412-415.
68. Sheldon, R.A. *Chirotechnology: Industrial Synthesis of Optically Active Compounds*; Marcel Dekker Inc.: New York, 1993; pp. 371-374.
69. Besse, P. and Veschambre, H. *Tetrahedron* **1994**, 50, 8885-8927.
70. Collman, J.P.; Zhang, X.; Lee, V.J.; Uffelman, E.S. and Brauman, J.I. *Science* **1993**, 261, 1404-1411.
71. Jacobsen, E.N. in *Catalytic Asymmetric Synthesis*; Ojima, I. Ed.; VCH: New York, 1993; pp. 159-202.
72. Holland H.L. *Organic Synthesis with Oxidative Enzymes*; VCH: New York, 1992; pp. 153-182.
73. Koch, A.; Reymond, J.L. and Lerner, R.A. *J. Am. Chem. Soc.* **1994**, 116, 803-804.
74. Neidleman, S.L.; Amon W.F., Jr. and Geigert, J. (Cetus Corp.) US 4284723 [*Chem. Abstr.* **1981**, 95, 167173].
75. Ortiz de Montellano, P.R. and Grab, L.A. *Mol. Pharmacol.* **1986**, 30, 666-669.
76. Ortiz de Montellano, P.R. and Grab, L.A. *Biochem.* **1987**, 26, 5310-5314.
77. Berkessel, A. lecture at the 6th international symposium on the activation of dioxygen and homogeneous catalytic oxidation, Noordwijkerhout, The Netherlands, April 17, 1996.
78. McCarthy, M.B. and White, R.E. *J. Biol. Chem.* **1983**, 258, 9153-9158.
79. Geigert, J.; Lee, T.D.; Dalietos, D.J.; Hirano, D.S. and Neidleman, S.L. *Biochem. Biophys. Res. Commun.* **1986**, 136, 778-782.
80. Ortiz de Montellano, P.R.; Choe, Y.S.; DePillis, G. and Catalano, C.E. *J. Biol. Chem.* **1987**, 262, 11641-11646.
81. Elfarra, A.A.; Duescher, R.J. and Pasch, C.M. *Arch. Biochem. Biophys.*, **1991**, 286, 244-251.
82. Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G. and Pasta, P. *Tetrahedron: Asymm.* **1993**, 4, 1325-1330.
83. Allain, E.J.; Hager, L.P.; Deng, L. and Jacobsen, E.N. *J. Am. Chem. Soc.* **1993**, 115, 4415-4416.
84. Zaks, A. and Dodds, D.R. *J. Am. Chem. Soc.* **1995**, 117, 10419-10424.
85. Lakner, F.J. and Hager, L.P. *J. Org. Chem.* **1996**, 61, 3923-3925.
86. Dexter, A.F. and Hager, L.P. *J. Am. Chem. Soc.* **1995**, 117, 817-818.
87. Dexter, A.F.; Lakner, F.J.; Campbell, R.A. and Hager, L.P. *J. Am. Chem. Soc.* **1995**, 117, 6412-6413.
88. Mashino, T.; Nakamura S. and Hirobe, M. *Tetrahedron Lett.* **1990**, 31, 3163-3166.
89. Blée, E. and Schuber, F. *Biochem. Biophys. Res. Commun.* **1990**, 173, 1354-1360.
90. Larrow, J.F. and Jacobsen, E.N. *J. Am. Chem. Soc.* **1994**, 116, 12129-12130.
91. Holland, H.L. *Organic Synthesis with Oxidative Enzymes;* VCH: New York, 1992; pp. 55-151.
92. Miller, V.P.; Tschirret-Guth, R.A. and Ortiz de Montellano, P.R. *Arch. Biochem. Biophys.* **1995**, 319, 333-340.
93. Sundaramoothy, M.; Terner, J. and Poulos, T.L. *Structure* **1995**, 3, 1367-1377.
94. Thomas, J.A.; Morris, D.R. and Hager, L.P. *J. Biol. Chem.* **1970**, 245, 3129-3134.
95. Geigert, J.; Dalietos, D.J.; Neidleman, S.L.; Lee, T.D. and Wadsworth, J. *Biochem. Biophys. Res. Commun.* **1983**, 114, 1104-1108.
96. van Deurzen, M.P.J.; van Rantwijk, F. and Sheldon, R.A. *J. Carbohydr. Chem.* in press.

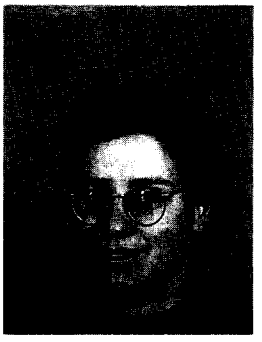
97. *Therapeutic Approaches to Inflammatory Diseases*; Lewis, A.J.; Doherty, N.S. and Ackerman, N.R. Eds.; Elsevier: New York, 1989; p. 229.
98. Szabó-Pusztay, K. and Szabó, L. *Synth. Commun.* **1979**, 276-277.
99. Inoue, Y.; Yamamoto, Y.; Suzuki, H. and Takaki, U. in *Developments in Selective Oxidations II*, Corés Corberán, V. and Vic Bellón, S. Eds.; Elsevier Science: Amsterdam, 1994; pp. 615-622.
100. Goehring, R.R.; Sachdeva, Y.P.; Pisipati, J.S.; Sleevi, M.C. and Wolfe, J.F. *J. Am. Chem. Soc.* **1985**, 107, 435-444.
101. RajanBabu, T.J.; Chenard, B.L. and Petti, M.A. *J. Org. Chem.* **1986**, 51, 1704-1712.
102. Quallich, G.J. and Morrisey, P.M. *Synthesis* **1993**, 51-53.
103. Crestini, C. and Saladino, R. *Synth. Commun.* **1994**, 24, 2835.
104. Corbett, M.D. and Chipko, B.R. *Biochem. J.* **1979**, 183, 269-276.
105. van Deurzen, M.P.J.; van Rantwijk, F. and Sheldon, R.A. *J. Mol. Cat. B: Enzymatic* **1996**, 2, 33-42.
106. Dawson, J.H. *Science* **1988**, 240, 433-439.
107. Du, P. and Loew, G.H. *Biophys. J.* **1995**, 68, 69-80.
108. Kitagawa, T. and Mizutani, Y. *Coord. Chem. Rev.* **1994**, 135/136, 685-735.
109. Poulos, T.L. and Kraut, J. *J. Biol. Chem.* **1980**, 255, 8199-8205.
110. Poulos, T.L. *Adv. Inorg. Biochem.* **1987**, 7, 1-36.
111 Vitello, L.B., Erman, J.E., Miller, M.A., Wang, J. and Kraut, J. *Biochemistry* **1993**, 37, 9807-9818.
112 Miller, M.A.; Shaw, A. and Kraut, J. *Struct. Biol.* **1994**, 1, 524-531.
113 Poulos T.K., *J. Biol. Inorg. Chem.* **1996**, 1, 356-359 and references cited therein.
114. Finzel, B.C.; Poulos, T.L. and Kraut, J. *J. Biol. Chem.* **1984**, 259, 13027-13036.
115. Piontek, K.; Glumoff, T. and Winterhalter, K. *FEBS Lett.* **1993**, 315, 119-124.
116 For a discussion of the role of the axial ligand see: Poulos T.K., *J. Biol. Inorg. Chem.* **1996**, 1, 356-359; Goodin, D.B., *J. Biol. Inorg. Chem.* **1996**, 1, 360-363; Banci, L.; Rosato, A. and Turano, P., *J. Biol. Inorg. Chem.* **1996**, 1, 364-367; Gross, Z., *J. Biol. Inorg. Chem.* **1996**, 1, 368-371; Rietjens, I.M.C.M.; Osman, A.M.; Veeger, C.; Zakharieva, O.; Antony, J.; Grodzicki, M. and Trautwein, A.X., *J. Biol. Inorg. Chem.* **1996**, 1, 372-376; Weiss, R.; Mandon, D.; Wolter., T., Trautwein, A.X.; Müther, M.; Bill, E.; Gold, A.; Jayaraj, K. and Terner, J., *J. Biol. Inorg. Chem.* **1996**, 1, 377-383.
117. Edwards, S.L.; Xuong, N.-H.; Hamlin, R.C. and Kraut, J. *Biochem.* **1987**, 26, 1503-1511.
118. Virion, A.; Courtin, F.; Dème, D.; Michot, J.L.; Kaniewski, J. and Pommier, J. *Arch. Biochem. Biophys.* **1985**, 24, 41-47.
119. Ohtaki, S.; Nakagawa, H.; Nakamura, M. and Yamazaki, I. *J. Biol. Chem.* **1982**, 257, 761-766.
120. Harvey, P.J.; Schoemaker, H.E. and Palmer, J.M. *FEBS Lett.* **1986**, 195, 242-246.
121. Schoemaker, H.E.; Lundell, T.K.; Hatakka, A.I. and Piontek, K. *FEMS Microbiol. Rev.* **1994**, 13, 321-332.
122. Wariishi, H.; Valli, K. and Gold, M.H. *Biochem. Biophys. Res. Commun.* **1991**, 176, 269-275.
123. Tuor, U.; Wariishi, H.; Schoemaker, H.E. and Gold, M.H. *Biochem.* **1992**, 31, 4986-4995.
124. Poulos, T.L. and Kraut, J. *J. Biol. Chem.* **1980**, 255, 10322-10330.
125. Ator, M.A. and Ortiz de Montellano, P.R. *J. Biol. Chem.* **1987**, 262, 1542-1551.
126. Ator, M.A.; David, S.K. and Ortiz de Montellano, P.R.*J. Biol. Chem.* **1987**, 262, 14954-14960.
127. Ortiz de Montellano, P.R.; David, S.K.; Ator, M.A. and Tew, D. *Biochem.* **1988**, 27, 5470-5476.
128. DePillis, G.D. and Ortiz de Montellano, P.R. *Biochem.* **1989**, 28, 7947-7952.
129. DePillis, G.D.; Wariishi, H.; Gold, M.H. and Ortiz de Montellano, P.R. *Arch. Biochem. Biophys.* **1990**, 280, 217-223.
130. Harris, R.Z.; Wariishi, H.; Gold, M.H. and Ortiz de Montellano, P.R. *J. Biol. Chem.* **1991**, 266, 8751-8758.
131. Ortiz de Montellano, P.R. and Kerr, D.E. *J. Biol. Chem.* **1983**, 258, 10558-10563.
132. Samokyszyn, V.M. and Ortiz de Montellano, P.R. *Biochem.* **1991**, 30, 11646-11653.
133. Poulos, T.L.; Edwards, S.L.; Wariishi, H. and Gold, M.H. *J. Biol. Chem.* **1993**, 268, 4429-4440.
134. Petersen, J.F.W.; Kadziola, A. and Larsen, S. *FEBS Lett.* **1994**, 339, 291-296.
135. Sundaramoothy, M.; Kishi, K.; Gold, M.H. and Poulos, T.L. *J. Biol. Chem.* **1994**, 269, 32759-32767.

136. Gross, Z. and Nimri, S. *J. Am. Chem. Soc.* **1995**, 117, 8021-8022.
137. Collman, J.P.; Kodadek, T.; Raybuck, S.A.; Brauman, J.I. and Papazian, L.M. *J. Am. Chem. Soc.* **1985**, 107, 4343-4345.
138. Ostovic, D. and Bruice, T.C. *J. Am. Chem. Soc.* **1989**, 111, 6511-6517.
139. Pérez, U. and Dunford, H.B. *Biochim. Biophys. Acta* **1990**, 1038, 98-104.
140. Pérez, U. and Dunford, H.B. *Biochem.* **1990**, 29, 2757-2763.
141. Nakamura, S.; Mashino, T. and Hirobe, M. *Tetrahedron Lett.* **1992**, 33, 5409-5412.
142. Hashimoto, S.; Tatsuno, Y and Kitagawa, T. *Proc. Natl. Acad. Sci. USA* **1986**, 83, 2417-2421.
143. P.R. Ortiz de Montellano in *Cytochrome P-450, Structure, Mechanism and Biochemistry*, P.R. Ortiz de Montellano, Ed.; Plenum Press: New York, 1986; p. 234.
144. Bagger, S. and Williams, R.J.P. *Acta Chem. Scan.* **1971**, 25, 976-982.
145. Arnao, M.B.; Hernandéz-Ruíz, J.; Varón, R.; García-Cánovas, F. and Acosta, M. *J. Mol. Cat. A: Chemical* **1995**, 104, 179-191.
146. Wilks, A. and Ortiz de Montellano, P.R. *J. Biol. Chem.* **1993**, 268, 22357-22362.
147. Tromp, M.G.M., Olafsson, G., Kren, B.E. and Wever, R. *Biochim. Biophys. Acta* **1989**, 1040 192-198.
148. Divi, R.L. and Doerge, D.R. *Biochem.* **1994**, 33, 9668-9674.
149. Baynton, K.J.; Bewtra, J.K.; Biswas, N. and Taylor, K.E. *Biochim. Biophys. Acta* **1994**, 1206, 272-278.
150. Ma, X. and Rokita, S.E. *Biochem. Biophys. Res. Commun.* **1988**, 157, 160-165.
151. Lambeir, A. and Dunford, H.B. *Eur. J. Biochem.* **1985**, 147, 93-96.
152. Wittenberg, J.B.; Noble, R.W.; Wittenberg, B.A.; Antonini, E.; Brunori, M. and Wyman, J. *J. Biol. Chem.* **1967**, 242, 626-634.
153. Cai, D. and Tien, M. *Biochem. Biophys. Res. Commun.* **1989**, 162, 464-469.
154. Nakajima, R.; Yamazaki, I. and Griffin, B.W. *Biochem Biophys. Res. Commun.* **1985**, 128, 1-6.
155. Yamazaki, H.; Araiso, T.; Hayashi. Y.; Yamada, H. and Makino, R. *Adv. Biophys.* **1978**, 11, 249-281.
156. Butler, A.; Clague, M.J. and Meister, G.E. *Chem. Rev.* **1994**, 94, 625-638.
157. Yamazaki, I. and Piette, L.H. *Biochim. Biophys. Acta* **1963**, 77, 47-64.
158. Tamura, M. and Yamazaki, I. *J. Biochem. (Tokio)* **1972**, 71, 311-319.
159. Chance, B.; Powers, L.; Ching, Y.; Poulos, T.; Schonbaum, G.R.; Yamazaki, I. and Paul, K.G. *Arch. Biochem. Biophys.* **1984**, 235, 596-611.
160. Shaanan, B. *J. Mol. Biol.* **1983**, 171, 31-59; Phillips, S.E.V. and Schoenborn, B.P. *Nature* **1981**, 292, 81-82. See also: Bytheway, I. and Hall, M.B. *Chem. Rev.* **1994**, 94, 639-658; Springer, B.A.; Sligar, S.G.; Olson, J.S. and Phillips, G.N., Jr. *Chem. Rev.* **1994**, 94, 699-714.
161 Pauling, L. and Coryell, C.D. *Proc. Natl. Acad. Sci. USA* **1936**, 22, 210-216.
162. Barr, D.P. and Aust, S.D. *Arch. Biochem. Biophys.* **1994**, 312, 511-515.
163. Chung, N. and Aust, S.D. *Arch. Biochem. Biophys.* **1995**, 316, 8151-8155.
164. van Deurzen, M.P.J.; Seelbach, K.; van Rantwijk, F.; Kragl, U. and Sheldon, R.A. *Biocatal. Biotransform.* in press.
165. Arnao, M.B.; Acosta, M.; del Río, J.A. and García-Cánovas, F. *Biochim. Biophys. Acta* **1990**, 1038, 85-89.
166. Hiner, A.N.P.; Hernández-Ruíz, J.; García-Cánovas, F.; Smith, A.T.; Arnao, M.B. and Acosta, M. *Eur. J. Biochem.* **1995**, 234, 506-512.
167. Adediran, S.A. *Arch. Biochem. Biophys.* **1996**, 327, 279-284.
168. Kohler, H.; Taurog, A. and Dunford, H.B. *Arch. Biochem. Biophys.* **1988**, 264, 438-449.
169. Vilter, H. *Phytochem.* **1984**, 23, 1387-1390.
170. de Boer, E.; van Kooyk, Y.; Tromp, M.G.M.; Plat, H. and Wever, R. *Biochim. Biophys. Acta* **1986**, 869, 48-53.
171. van Schijndel, J.W.P.M.; Vollenbroek, E.G.M. and Wever, R. *Biochim. Biophys. Acta* **1993**, 1161, 249-256.
172. van Schijndel, J.W.P.M.; Barnett, P.; Roelse, J.; Vollenbroek, E.G.M. and Wever, R. *Eur. J. Biochem.* **1994**, 225, 151-157.
173. Everett, R.R.; Kanofsky, J.R. and Butler, A. *J. Biol. Chem.* **1990**, 265, 4908-4914.

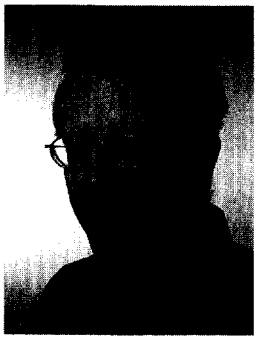
174. de Boer, E.; Plat, H.; Tromp, M.G.M.; Wever, R.; Franssen, M.C.R.; van der Plas, H.C.; Meijer, E.M. and Schoemaker, H.E. *Biotechnol. Bioeng.* **1987**, 30, 607-610.
175. de Boer, E.; Boon, K. and Wever, R. *Biochem.* **1988**, 27, 1629-1635.
176. Butler, A. in *Bioinorganic catalysis*; Reedijk, J. Ed.; Marcel Dekker: New York, 1993; pp. 425-445.
177. Messerschmidt, A. and Wever, R. *Proc. Natl. Acad. Sci. USA* **1996**, 93, 392-396.
178. Bantleon, R.; Altenbuchner, J. and van Pée, K.-H. *J. Bacteriol.* **1994**, 176, 2339-2347.
179. Weng, M.; Pfeifer, O.; Krauss, S.; Lingens, F. and van Pée, K.-H. *J. Gen. Microbiol.* **1991**, 137, 2539-2546.
180. Itoh, N.; Morinaga, N. and Kouzai, T. *Biochim. Biophys. Acta* **1994**, 1207, 208-216.
181. Hecht, H.J.; Sobek, H.; Haag, T.; Pfeifer, O. and van Pée, K.-H. *Struct. Biol.* **1994**, 1, 532-537.
182. Björkling, F.; Godtfredsen, S.E. and Kirk, O., *J. Chem. Soc., Chem. Commun.* **1990**, 1301-1303; Kirk, O.; Björkling, F. and. Godtfredsen, S.E. (Novo Nordisk A/S), PCT Int. Appl. WO 91 04333; Björkling, F.; Frykman, H.; Godtfredsen, S.E. and Kirk, O., *Tetrahedron* **1992**, 48, 4587-4592; de Zoete, M.C.; van Rantwijk, F.; Maat, L. and Sheldon, R.A., *Recl. Trav. Chim. Pays-Bas* **1993**, 112, 462-463.
183. Flohé, L. in *Glutathione, Chemical, Biochemical and Medical Aspects*; Dolphin, D.; Avramovic, O. and Poulsen, R. Eds.; Wiley: New York, 1989; pp. 643-731.
184. Ball, A.S. and Trigo, C. *Biochem. Soc. Trans.* **1995**, 23, 272-276.
185. Black, M.; Lin, L. and Guthrie, J. (Medisence, Inc.) PCT Int. Appl. WO 94 27140 [*Chem. Abstr.* **1995**, 122, 182729].
186. Tamaki, H.; Kizaki, T. and Ichikura, S. (Toray Industries) JP 07270414 [*Chem. Abstr.* **1996**, 124, 81501].
187. Yamazaki, M. (Konishiroku Photo Ind.) JP 07306203 [*Chem. Abstr.* **1996**, 124, 255269].
188. Harazono, K.; Kondo, R.and Sakai, K. *Appl. Environ. Microbiol.* **1996**, 62, 913-917.
189. Damhus, T. and Petersen, D.A. (Novo Nordisk A/S.) PCT Int. Appl. WO 95 33039 [*Chem. Abstr.* **1996**, 124, 205673].
190. Lui, D.K.K. and Convents, A.C. (Procter and Gamble Co.) US 5451337 [*Chem. Abstr.* **1996**, 124, 59946].
191. Johnson, M.A. and Pokora, A.P. (Mead Corp.) US 5370770 [*Chem. Abstr.* **1995**, 122, 317211].
192. Allen, R.C. (Exoxemis Inc.) Eur. Pat. Appl. EP 500387 [*Chem. Abstr.* **1992**, 117,184847].
193. Barnett, P.; Hondmann, D.H.; Simons, L.H.; ter Steeg, P.F. and Wever, R. (Unilever N.V.; Unilever PLC) PCT Int. Appl. WO 95 27046 [*Chem. Abstr.* **1996**, 124, 4003].
194. Pellico, M.A. US 5453284 [*Chem. Abstr.* **1995** 123, 265827].
195. Carmichael, R.; Fedorak, P.M. and Pickard, M.A. *Biotechnol. Lett.* **1985**, 7, 289-294.
196. Cooper, V.A.; Nicell, J.A. *Water Res.* **1996**, 30, 954-964.
197. Junino, A.; Andrean, H.; Tuloup, R. and Higgins, I.J. (Oreal S.A.) Eur. Pat. Appl. EP 441689 [*Chem. Abstr.* **1992** 116, 66915].
198. Snelting, H. *Chemisch Magazine* **1995**, 6, 264.
199. Andersen, H.D.; Jensen, E.B. and Welinder, K.G. (Novo Nordisk A/S) Eur. Pat. Appl. EP 505311 [*Chem. Abstr.* **1993**, 118, 74769].
200. Pickard, M.A.; Tenshuk, T.A.; Kadima, A. and Carmichael, R.D. *J. Ind. Microbiol.* **1991**, 7, 235-242.
201. Polvinen, K.; Lehtonen, P.; Leisola, M. and Visuri, K. in *Enzymes in Biomass Conversion*; Leatham, G.F. and Himmel, M.E. Eds.; Am. Chem. Soc.: Washington, 1991; pp 225-235.
202. Pedersen, A.H.; Vind, J.; Svendsen, A.; Cherry, J.R.; Lamsa, M.; Schneider, P. and Jensen, B.R. (Novo Nordisk A/S) PCT Int. Appl. WO 95 10602 [*Chem. Abstr.* **1995**, 123, 173621].
203. van Deurzen, M.P.J.; Groen, B.W.; van Rantwijk, F. and Sheldon, R.A. *Biocatalysis* **1994**, 10, 247-255.
204. Seelbach, K.; van Deurzen, M.P.J.; van Rantwijk, F.; Sheldon, R.A. and Kragl., U. *Biotechnol Bioeng.* submitted for publication.

(*Received* 22 *April* 1997)

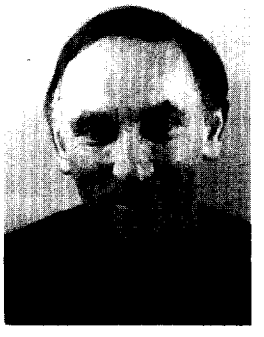
Biographical Sketch



Marion P. J. van Deurzen



Fred van Rantwijk



Roger A. Sheldon

Marion van Deurzen, born 1968, completed her studies at the Eindhoven University of Technology. She moved to the Delft University of Technology to work on the synthetic application of peroxidases, under supervision of Prof. R.A. Sheldon. She received her PhD in 1996 and is currently with the Unilever Research Laboratory in Vlaardingen.

Fred van Rantwijk (1943) studied organic chemistry at the Delft University of Technology where he remained as a staff-member. He received his Ph.D. in 1980 under the direction of Professor H. van Bekkum. Since the late eighties he works on the application of enzymes in organic synthesis.

Roger Sheldon was born in Nottingham (UK) in 1942. He obtained a PhD in organic chemistry (1967) from the University of Leicester (UK) under the joint supervision of S. Trippett and R.S. Davidson. Following post-doctoral studies with Jay Kochi in the US on reactions of metal ions with free radicals, he joined Shell Research in Amsterdam in 1969 where he carried out research on various catalytic processes, particularly liquid phase oxidations. From 1980 to 1990 he was R&D Director of Andeno (a subsidiary of DSM) in Venlo (Netherlands). In 1991 he moved to his present position as Professor of organic chemistry and catalysis at the Delft University of Technology (Netherlands). His research interests encompass homogeneous and heterogeneous catalysis of liquid phase oxidations, organometallic catalysis in aqueous media and enzymatic conversions under non-natural conditions.