Chapter 24

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CHAPTER 24

Vanadium Chloroperoxidases as Versatile Biocatalysts

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24.1 Introduction

The vanadate-dependent haloperoxidases are enzymes that catalyse a rather simple reaction – the two-electron oxidation of a halide by hydrogen peroxide resulting in the formation of hypohalous acids, according to eqn (24.1).

$$Cl^- + H_2O_2 + H^+ \longrightarrow HOCl + H_2O \quad (24.1)$$

These hypohalous acids are very reactive and are able to halogenate a broad range of organic substrates. In the absence of organic compounds, the formed HOX will non-enzymatically react with the substrate hydrogen peroxide resulting in the formation of singlet oxygen ($^1O_2$, eqn (24.2). The rate of the non-catalytic reaction between HOCl and H_2O_2 is pH dependent and decreases strongly at low pH (<7).[1]

$$HOCl + H_2O_2 \longrightarrow {}^1O_2 + H_2O + HCl \quad (24.2)$$

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Haloperoxidases able to catalyse the oxidation of chloride, bromide or iodide are named chloroperoxidases and enzymes able to oxidise bromide and iodide are characterised as bromoperoxidases. The iodoperoxidases are only able to oxidise iodide and, thus, will not be discussed here. Since a bromoperoxidase may also oxidise chloride, albeit with low specificity constant, the distinction between the haloperoxidases is somewhat arbitrary. The vanadium bromo- and iodoperoxidases (VBPO's, VIPO's) are mostly found in the marine environment (macro-algae and cyanobacteria) and are involved in the production of large amounts of brominated and iodinated compounds that are released in the marine environment.[2,3] These BPO's produce oxidised bromine species (HOBr, Br_2 and Br_3^-) that will react selectively with a range of organic molecules and organic matter.[4–6] However, there is some evidence that some VBPO's from red seaweeds are selective since they show diastereoselectivity in the bromohydrin formation.[7,8] The VBPO's are highly stable and the peroxidases from the brown seaweed *Ascophyllum nodosum* have been used in the biosynthesis of brominated compounds. The many properties and successful use of this enzyme in bromination reactions are described in detail elsewhere[9,10] and will only be discussed here shortly.

The vanadium chloroperoxidases (VCPO's) are especially interesting because of their ability to oxidise Cl^-. They are found in a group of terrestrial fungi, the so-called dematiaceous hyphomycetes belonging to the phylum *Ascomycota*. These fungal VPO's probably function in the decay of plant debris and have a possible role in the degradation of the cell walls of the plant host via the formation of HOCl.[11] These enzymes just release HOCl in solution and there is no evidence for regio- or stereoselective halogenation catalysed by these fungal VCPOs.

In contrast, the vanadium peroxidases NapH1 and Mcl24 from the marine prokaryote *Streptomyces* bacteria *Streptomyces* sp. CNQ-525 and *Streptomyces* sp. CNH-189, respectively, catalyse stereoselective halogenation of merochlorin and napyradiomycin molecules. These enzymes do not form free diffusible HOCl as judged from their inability to chlorinate monochlorodimedone, a widely used assay[10] to asses halogenating activity, but instead are able to specifically catalyse the stereoselective chlorination-cyclisation of their natural substrates. This suggests a very specific binding of the substrate to the active site of these enzymes. A detailed description of the properties of these enzymes and their dedicated role in the biosynthetic route to the napyradiomycin family of bacterial meroterpenoids can be found elsewhere.[12,13]

There are a number of other enzyme classes that are able to carry out halogenations reactions and are, or maybe become, synthetically useful. These are the heme-dependent haloperoxidases, the non-heme α-keto glutarate-dependent halogenases and the flavin-dependent halogenases. This chapter focuses on the use of the fungal VCPOs that are more stable than enzymes from the other classes in biocatalytic halogenation reactions. For information on these classes, the reader is referred to several reviews.[14–16]

24.2 Fungal Vanadium Chloroperoxidases

Many mechanistic and molecular details are available for vanadium chloroperoxidases. Most studies have been done with the enzyme from the fungus *Curvularia inaequalis*, though enzymes from other species have also been studied, *e.g.*, those from *Drechslera biseptata*[17] and *Embellisia didymospora.*[18] This includes detailed structural data of the enzyme,[19] the nature of the active site, the nature of the peroxido intermediate that is formed during catalysis[20] and the role of the various amino acids ligated and coordinated to the vanadate.[21,22]

24.2.1 Stability of the VCPO

Early on it was discovered that the fungal VCPO is unusually stable, like the VBPOs from the marine environment. The enzyme can be stored for weeks in water-miscible and immiscible solvents such as methanol, ethanol, 2-propanol or ethylacetate,[23,24] and also remains active when used as a cosolvent.[24] The enzyme is also resistant towards chaotropic agents. For example, at incubation in guanidine-HCl only at a concentration of 2 M the enzyme starts to lose activity[23] and at 3.7 M, only half the original activity is lost. At these concentrations most enzymes have lost their activity completely. Also, the VCPO remains catalytically active in a polydisperse nonionic surfactant, for at least 10 h, converting H_2O_2 into a constant flow of $^1O_2$.[25] The thermostability as shown in Figure 24.1 is also very high and close to that of thermostable enzymes. The midpoint temperature of denaturation of the CPO's *from Curvularia inaequalis* and *Drechslera biseptata* are about 90 and 82 °C, respectively.

These midpoint temperatures of thermal denaturation are close to those found for thermophilic enzymes. The enzyme structure of VCPO from *C. inaequalis* is mainly α helical with two four-helix bundles and some antiparallel beta sheets. The high stability is probably due to the very compact packing of the helices and the hydrophobic interaction in the middle of these bundles. There are no disulphide bridges.[19]

Currently five VHPO crystal structures are available[13,15] and the active site in all these enzymes consists of negatively charged vanadate held in place by positively charged amino acids lysine and two arginines. Further, there is a covalent bond to the $N\varepsilon_2$ of a histidine and two hydrogen bonds to a serine and a glycine residue. Finally, there is a hydrogen bond of the axial oxygen atom to the $N\varepsilon_1$ atom of a histidine. The active site, as illustrated in Figure 24.2, is conserved in all vanadium haloperoxidases known to date, with some minor differences.

Thus, the vanadium enzymes differ significantly in their active site structure from the well-known heme peroxidases. These enzymes and their prosthetic group the oxido-metallate are very resistant towards oxidative inactivation by both substrate (hydrogen peroxide) and products (hypohalous acids and singlet oxygen) as will be discussed in some detail. This

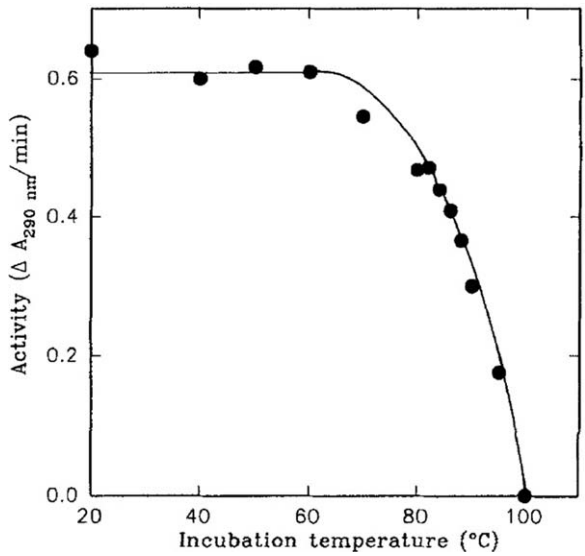

Figure 24.1 The thermostability of VCPO. Samples of VCPO were incubated for 5 min in Tris-buffer (pH 8.3) after which time the chlorinating activity was measured. Prolonged incubation for 15 min did not affect the midpoint temperature.
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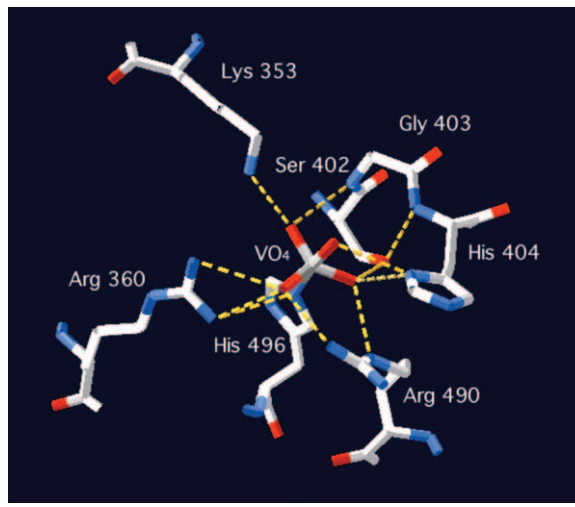

Figure 24.2 The active site of VCPO from Curvularia inaequalis (PDB accession number 11DQ).
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resistance towards inactivation[23–27] is in strong contrast to heme-containing enzymes which are rapidly inactivated even at micromolar concentrations presumably by the oxidation of the labile cofactor.

24.2.2 Kinetic Properties Vanadium Chloroperoxidase from *Curvularia inaequalis*

This enzyme shows a pH optimum in the bromination or chlorination varying from pH 5 to 5.5 depending on the concentration of hydrogen peroxide, the halide used and its concentration.[28] Steady-state kinetic studies[23] on the oxidation of chloride by VCPO reveal a mechanism in which hydrogen peroxide binds first to the enzyme forming a peroxido intermediate which oxidises the halide. High halide concentrations inhibit the enzyme strongly whereas high peroxide concentrations have hardly any effect.[23,29] This substrate inhibited Bi Bi Ping-Pong mechanism resembles that of the VBPOs. At pH values below the pH optimum the chlorinating activity is inhibited by chloride whereas higher pH values chloride oxidation follows a Michaelis–Menten type of curve. As Table 24.1 shows, the K_m value for chloride oxidation is strongly dependent on pH and its value increases nearly linearly with pH. The kinetics of bromide oxidation is similar to that of the chloride oxidation.[23,29]

However, the K_m for bromide in the order of μMolar is surprisingly small (<10 μM) in the pH range 4.2–6.3 and it is much less dependent upon pH. Bromide is much more inhibitory than chloride.[29] This should be kept in mind when applying the enzyme in synthetic halogenation procedures. It should be noted that the turnover frequencies (k_{cat} values) mentioned in Table 24.1 have been obtained for pure enzyme and are derived from V_{max} values from Lineweaver–Burk plots. These values are in general significantly higher than those obtained at a fixed halide and H_2O_2 concentrations. Table 24.1 also shows that the K_m values for H_2O_2 are in the μMolar range. Inhibition by H_2O_2 even at high concentrations has not been reported so, again, in preparative purposes the H_2O_2 concentration is not crucial. The specificity constants (k_{cat}/K_m) for the oxidation of Br^- are about 5–10-times

Table 24.1 Kinetic parameters of the oxidation of Br^- and Cl^- catalysed by VCPO.

		X^-			H_2O_2		
Halide	pH	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}\,s^{-1}$)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1}\,s^{-1}$)
Br^- [21,30]	4.2	<5	253	$>5.1\times10^7$	90	250	2.6×10^6
	5.2	9	248	2.8×10^7	35	203	5.8×10^6
	6.3	7	37	5.3×10^6	<5	33	6.6×10^6
	8.0	120	1	8.3×10^3	<5	1	$>2\times10^5$
Cl^- [23,29]	5.0	1.1×10^3	23	2.0×10^4	49	183	3.7×10^6
	6.3	1.0×10^4	7.3	7.1×10^2	2.6	152	5.8×10^6
	8.0	1.1×10^5	n.d.	n.d.	n.d.	n.d.	n.d.

higher than those for chloride oxidation, in part reflecting the ease of oxidation of Br^-.

24.2.3 Use of the VCPO in the Production of Singlet Oxygen

In fact, the VCPO can be used as a highly efficient catalyst for the production of singlet oxygen[27] under mildly acidic conditions according to eqn (24.1) and (24.2). In these reactions the halide is regenerated and the process is catalytic, resulting in slow and controlled formation of $^1O_2$. To minimise the side reactions of $^1O_2$ with compounds other than HOCl, high H_2O_2 concentration (0.1 M–1.0 M) should be used.

Figure 24.3 illustrates the surprising stability of VCPO against 0.5 M H_2O_2 under continuous turnover producing $^1O_2$ for at least one hour in the presence of 5 mM chloride. This system under appropriate conditions may function as efficient catalytic system for the generation of $^1O_2$ under slightly acidic conditions.[27] Chloride should be used as substrate rather than bromide since with the latter formation of side products is observed. The VBPO from *Ascophyllum nodosum* has also been tested as singlet oxygen producer. Unlike the VCPO, this enzyme though it initially produces singlet oxygen, is inactivated in time. Using anthracene-9,10-bis(ethane sulphonate) as a singlet oxygen trap full conversion is observed into the corresponding endoperoxide. During the conversion[27] the enzyme activity is not affected for 25 000 turnovers within 75 minutes with a turnover frequency of 5.6 s^{-1}. The rate is about 10^4-times higher than those based on Na_2MoO_4 or $La(NO_3)_3$/NaOH and which are not effective at slightly acidic pH values. The enzyme is also active in singlet oxygen formation in non-ionic micro-emulsions and

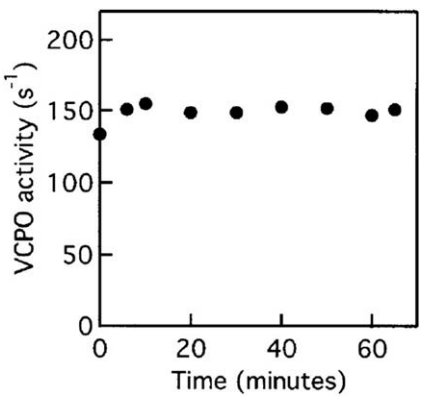

Figure 24.3 Resistance of the VCPO against inactivation by H_2O_2 and $^1O_2$. The formation of $^1O_2$ was initiated by the addition of 0.5 M H_2O_2 and 5 mM Cl^-. At selected time intervals samples were taken and the brominating activity of the VCPO assayed.
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fully stable for at least 10 h converting H_2O_2 in a constant flow of $^1O_2$.[25] This makes the enzyme a viable alternative for inorganic systems.

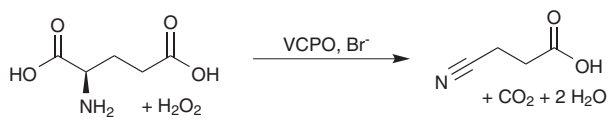
24.2.4 Use of VCPO in Decarboxylation of Amino Acids

Nitriles are important building blocks in the synthesis of a variety of pharmaceuticals, in polymer production or as industrial solvents such as acetonitrile. Many methods to produce them are available but they are either energy-intensive or use toxic chemicals (*e.g.*, HCN) and there is clearly a need for greener alternatives. In this respect, VCPO may be a viable alternative. When the amino acid phenylalanine and glutamate are exposed to the enzyme in the presence of Br^- and H_2O_2 at pH 5.6 the amino acids are decarboxylated[31] resulting in the formation of the corresponding nitriles (phenylacetonitrile and 3-cyanopropanoic acid). The amino group of the amino acid reacts with HOBr forming a N-bromo-intermediate that decays to 3-cyanopropanoic acid and CO_2 (Scheme 24.1).

This scheme shows that Br^- is regenerated and thus the reaction is catalytic in bromide. Since the K_m for bromide oxidation is very small (<10 μM, see Table 24.1) only a catalytic amount of bromide is needed. Other amino acids are also converted by this enzymatic system, *e.g.*, phenylalanine into phenylacetonitrile. However, in this conversion not only phenylacetonitrile is formed but also the corresponding aldehyde as a side product. This oxidative decarboxylation catalysed *via* formation of HOBr may have a potential application in the valorisation of biomass that is rich in glutamic acid.[31]

24.2.5 Sulphoxidation by VBPO and VCPO

Optically active sulphoxides are important synthons in particular for the preparation of biologically active compounds and they also function as carriers of chiral information in asymmetric transformations. No general method is available to synthesise these compounds. Several oxidoreductases catalyse the enantioselective sulphoxidation of a variety of sulphides and also the VBPO's have been successfully used in this reaction[10] though the k_{cat} values of enantioselective sulphoxidations (1 min^{-1}) are in general much slower than the k_{cat} of the brominating activity (166 s^{-1}) of for example the VBPO from *A. nodosum.*[32] In contrast to VBPO, VCPO is not capable of a direct and selective oxygen transfer to methyl phenyl sulphide and a racemic mixture is formed. It has been suggested[33] that the aromatic sulphide is directly oxidised by the very reactive peroxido enzyme intermediate by one



Scheme 24.1 Enzymatic oxidative decarboxylation of glutamic acid into 3-cyanopropanoic acid by vanadium chloroperoxidase from *C. inaequalis*.

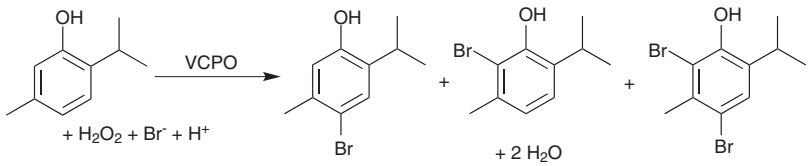
electron and the formed positively charged sulphur radical decays to the racemic sulphoxide.

24.2.6 Halogenation of Phenolics and Alkenes by Haloperoxidases

Chemical halogenation of organic compounds by elementary halogens or hypohalous acids suffers from the high reactivity of the reagents leading to unwanted side products and the direct use of these reagents requires special non-corrosive equipment. *N*-halocompounds as halogenating agents may also be used but these need to be halogenated as well, causing significant organic waste. Similarly, vanadium-based catalysts may be used,[34] but in general the turnover is low. Transition metals such as W and V and vanadium peroxides have been shown to be useful in the conversion of olefins to halohydrines.[35–37] The best method from a green-chemistry perspective would be the generation of halogenating species from halides by the oxidant H_2O_2 or O_2.[38] Alternatively oxone[39] (potassium monopersulphate) in the presence of alkali metal bromides may be used to obtain brominated compounds. Given the importance of organohalogens, the use of enzymes able to halogenate compounds under mild conditions is becoming more and more attractive since the process may be greener and more selective. Several studies and reviews have appeared[14,40–43] in which the application of the heme chloroperoxidase form *Caldariomyces fumago* in halogenation of several compounds is discussed. The popularity of using this enzyme in conversions is mainly due to the fact that it is commercially available and its initial high turnover frequency (1000 s^{-1}) but it is rapidly inactivated by moderate peroxide concentrations.[44] In addition, the activity of the heme CPO is readily eliminated at temperatures above 50 °C and storage at slightly elevated temperatures leads to inactivation.[45]

Alternatively, the VBPO from *Ascophyllum nodosum* can be used in the synthesis of brominated organic compounds and several studies have appeared[10] of the successful application of this enzyme in the synthesis of brominated phenols, barbituric derivatives and pyrroles. In particular, the controlled and slow generation of HOBr in solution allows more specific bromination of a susceptible site on the organic substrate.[46] However, though the VBPO may be purified in pretty large amounts[10] from its natural source (*e.g.*, the seaweed *Ascophyllum nodosum)* this source is not universally accessible. To date, successful methods to express this enzyme or other VBPO's in sufficient amounts from seaweeds have failed due to the formation of insoluble inclusion bodies from which it is difficult to obtain active enzyme. Also, this VBPO is only moderately resistant towards oxidative inactivation by H_2O_2.[27,47]

A much better alternative may be the VCPO considering its superb stability and the fact that it can easily expressed in sufficient amounts in convenient hosts.[21,30,48] Recently the VCPO was also successfully His-tagged without any effect on known enzymatic parameters (unpublished results). Despite this



Scheme 24.2 Bromination of thymol to 2-bromo, 4-bromo and 2,4-dibromothymol by VCPO.

only a few studies have appeared on the use of the enzyme in conversions. To investigate the feasibility of the VCPO in bromination and chlorination reactions, the electrophilic halogenation of thymol was chosen as model substrate.[24] Since the kinetic properties of the VCPO towards bromide or chloride (Table 24.1) differ it is difficult to compare the efficiency and specificity at the same pH or substrate concentrations. For example, it was observed that when using 10 mM of each halide at pH 5 and dosing the incubation every 30 min for 6 h with 1 mM H_2O_2 complete conversion of the thymol in brominated compounds was observed (see Scheme 24.2) whereas when chloride was used the conversion was only 50%. In this bromination reaction an apparent turnover frequency of 55 s^{-1} was found.

This bromination process is kinetically controlled. In the initial stage of the reaction first 2-bromo- and 4-bromo thymol are formed which are subsequently converted into the 2,4-dibromothymol. Up-scaling the process to obtain synthetic useful amounts is hampered by the fact that high bromide concentrations (>10 mM) inhibit the reaction[29] and bromide was added continuously to the incubation. Further thymol poorly dissolves in water and 50% of ethanol had to be used as co-solvent. This semi-preparative method yielded 0.09, 0,69 and 0.14 g of 2-bromo-,4-bromo- and 2,4-dibromothymol, respectively. The turnover frequencies obtained[24] are high, up to 55 s^{-1} and close to the maximum value of 250 s^{-1} that can be obtained for the enzyme under optimal conditions (see Table 24.1). A total turnover number of 2×10^6 can be calculated from the data. This illustrates the robustness of this enzyme and its insensitivity to hydrogen peroxide which makes this enzyme a better catalyst than the heme-containing chloroperoxidase from *Caldariomyces fumago* for which the concentration of added hydrogen peroxide has to be carefully controlled,[40] and an even better catalyst than the vanadium bromoperoxidase.[46]

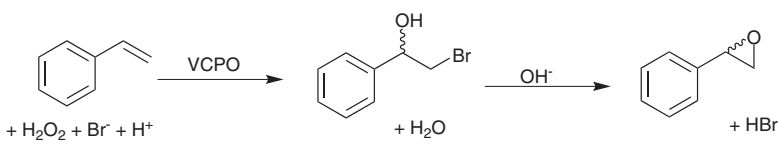
24.2.7 Formation of Halohydrins and Epoxides

Halohydrins are useful intermediates in the synthesis of more complex compounds and can easily be converted into epoxides. Classical chemical methods to obtain halohydrins suffer from the formation of side products and the corrosive conditions that have to be used. Haloperoxidases have been used early on[49] to produce α,β halohydrins on an analytical scale from gaseous alkenes (ethylene, propylene, butane-1, butane 2, isobutylene,

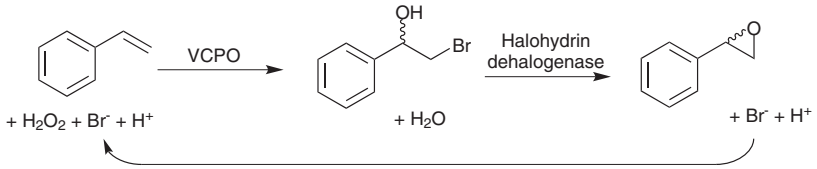
butadiene and allene) using the heme CPO from *Caldariomyces fumago* and bovine lactoperoxidase. These enzymes are very susceptible to hydrogen peroxide and the concentration used (30 mM) prevented long incubation times and sufficient yields. In 1988 the formation of the bromohydrins by a peroxidase[50] – later shown to be a VBPO[51] from the red seaweed *Corallina pilulifera* – was reported. Substrates were substituted alkenes, including cyclohexene, styrene, transcinnamyl alcohol, trans-cinnamic acid and *cis*-propenylphosphonic acid. The results showed that the products formed result from hypohalous addition across the carbon–carbon bond forming a bromonium intermediate. As shown in Section 24.1.7, the use of vanadium CPO may be a much better biocatalytic alternative and this was evaluated[52] using the halohydroxylation of styrene as a model reaction (see Scheme 24.3).

Indeed, full conversion and formation of bromohydrin were obtained at the pH optimum of the enzyme (pH 5) with only traces of side products. When chloride was used as a substrate, the corresponding chlorohydrin was formed. As expected from a process in which the enzyme produces HOBr or HOCl, enantioselectivity is not observed. At more alkaline pH values more epoxide is formed in line with the base-catalysed intramolecular ring closure. In the presence of 170 mM hydrogen peroxide and 160 mM KBr 40 mM water soluble styrene-4-sulphonate was easily converted within 6.5 h into it the corresponding bromohydrin by 0.1 μM VCPO. The data showed that the VCPO performed 0.4×10^6 catalytic turnovers which could be increased to 1.3×10^6 by increasing the concentration of both the styrene-4-sulphonate and the KBr concentrations. The turnover frequency initially was 69 s^{-1} but decreased to 15 s^{-1} at a later stage of the reaction. The values of these kinetic parameters are close to those obtained in bromination of thymol by VCPO. The substrate scope of the enzymatic halogenation is large. In the presence of ethanol as a co-solvent for the poorly soluble substrates, various aromatic and aliphatic alkenes were converted into the corresponding halohydrins with satisfactory yields (60–88%). In general, at the same halide concentration the yields with bromide are higher. Preparative scale (1.1 g) reactions of some alkenes were also reported.[52]

Transformation of the formed halohydrins into the corresponding epoxides is also possible. This ring closure occurs spontaneously at alkaline pH. However, the VCPO (pH optimum 5) is only active at slightly acidic pH values. A two-step procedure was carried out[52] in which first the halohydrin is formed enzymatically followed by a pH increase to pH 10. This approach was successful for most of the aromatic and aliphatic alkenes.



Scheme 24.3 Enzymatic formation of α,β-bromohydrin from styrene.



Scheme 24.4 Enzymatic cascade for the conversion of alkenes into epoxides.

An enzymatic cascade in a one-pot procedure was also developed[52] using the halohydrin dehalogenase that will catalyse the ring closure at more neutral pH values (pH 7, see Scheme 24.4). Only 33% of the styrene was converted into the epoxide. However, the low yield is due to a mismatch of the pH optima, the dehalogenase has a pH optimum of 8–9 whereas that of VCPO is 5–5.5. Combining a halohydrin dehalogenase with haloperoxidase to obtain epoxides *via* an alternate pathway has been proposed before.[49] However, details on this study are lacking.

This enzymatic cascade has the advantage that bromide is regenerated in the reaction and, thus, catalytically present.

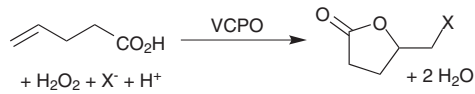
The group of Dordick[53] reported a very peculiar reaction. VCPO from *C. inaequalis* was shown to catalyse the hydroxylation, bromination and demethylation of 2,4,6-trinitrotoluene (TNT) in the presence of H_2O_2 and Br^- under alkaline conditions (pH 8). This is a surprising result considering the pH optimum of the enzyme (5–5.5) and the very low brominating activity at pH 8 (1 s^{-1}, See Table 24.1). No follow-up study has been reported.

24.2.8 Haloetherification by Bromoperoxidases

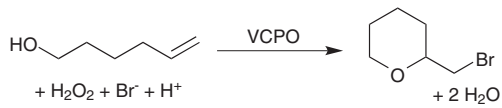
Using the VBPO's from marine red algae it was shown that the monoterpene nerol and sesquiterpene (*E*)-(+)-nerolidol were brominated and cyclised to a cyclic ether.[54,55] However, the yield (5%) was low and bromohydrins and epoxides were also formed. It is likely the enzymes form a bromonium ion that reacts at the alkene to form a bromonium intermediate, which, by a nucleophilic reaction with a nearby alcohol group, results in a cyclic bromoether structure.

24.2.8.1 Halolactonisation of Unsaturated Carboxylic Acids by VCPO

Bromolactonisation has been studied[56–58] in the past using the heme lactoperoxidase from milk and a bromoperoxidase (BPO) from *Laurencia nipponica*. In these studies, it was shown that these enzymes catalysed the intramolecular cyclisation of (3*E*)- and (3*Z*)-laurediol to the 8-membered bromoethers (*E*)-deacetyllaurencin and (*Z*)-prelaureatin. However, the yields of brominated cyclic lactones were very low. Bromolactonisation of 4-pentynoic acid and formation of a brominated furanone using the VBPO from the red alga *Delisea pulchra* was also reported.[59]



Scheme 24.5 Halolactonisation of 4-pentanoic acid into 5-(bromomethyl)dihydro-2(3*H*)-furanone.



Scheme 24.6 The etherification 40 mM of 5-hexen-1-ol into 2-(bromomethyl)tetrahydro-2*H*-pyran.

Recently it was shown that γ,δ-unsaturated carboxylic could also be halocyclised by VCPO.[48] Using 4-pentanoic acid as a substrate (Scheme 24.5) the corresponding halolactones could easily be obtained.

It was possible to prepare 0.91 and 1.4 g of the chloro-and bromolactone products from 10 mMol 4-pentenoic acid corresponding to 70% and 80% isolated yield. Also, a number of γ,δ unsaturated carboxylic acids were converted into the corresponding brominated or chlorinated cyclic lactones. As could be expected, the reaction proceeded optimally at pH 5. In most cases the conversion was 100%.

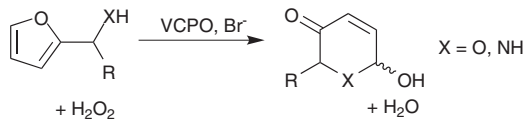
24.2.8.2 Conversions of Alkenols into Haloethers by VCPO

Haloetherification was also studied[48] using 5-hexen-1-ol as a substrate (see Scheme 24.6) and could be converted into 36 mM 2-(bromomethyl)tetrahydro-2*H*-pyran within 24 h.

This corresponds to a total turnover number of 360 000. It was possible to convert several other alkenols into the corresponding chloro- or bromoethers with good selectivity. The potential environmental benefits of the enzymatic process compared to the chemical proces using bromosuccinimide as a brominating agent were also demonstrated.[48] The mass intensities are comparable, but the chemical process yielded more waste.

24.2.9 Achmatowicz Reaction Catalysed by VCPO

The Achmatowicz reaction in which α-heterosubstituted furfural derivatives are oxidatively converted into six-membered O- or N-heterocyclic building blocks gives access to range of useful building blocks. In this reaction the furan ring is oxidatively activated using for example *m*-chloroperbenzoic acid or bromine.[60,61] Also biocatalytic conversions have been reported. For example, the heme chloroperoxidase from *Caldariomyces fumago* has been used in the selective oxidation of furfuryl alcohols.[62] However, the supply of H_2O_2 has to be carefully tuned in order to avoid oxidative inactivation of the



Scheme 24.7 The (aza)-Achmatowicz conversion mediated by VCPO.

peroxidase. The very robust VCPO has been exploited[63] in an (aza)-Achmatowicz reaction in which functionalised piperidinones or substituted pyranones are formed (see Scheme 24.7).

As expected, the oxidation of furfural derivatives proceeded optimally at pH 5 and for the aza-Achmatowicz reaction to proceed only low amounts of bromide were needed. This prompted the group[63] to carry out the reaction in seawater which was buffered 1 : 1 with 0.1 M citrate buffer (pH 5) as a source of bromide. Indeed, conversion of *N*-Boc protected 3-aminofuran was observed though the rate was significantly slower than in defined buffers. This was attributed to the high concentration of chloride in seawater and its inhibitory effect. Several furanyl derivatives[63] were fully converted into substituted piperidinones and prepared on a semi-preparative scale (about 0.2 g) using a reaction medium of ethanol with 100 mM citrate buffer at pH 5 [1 : 1 (v/v)] to solubilise the substrates. The turnover frequency was 8.7 s^{-1} and the total turnover number was larger than 10^6. It was also possible to obtain a substituted pyranone *via* the Achmatowicz rearrangement of a furfural alcohol. The use of VCPO in these rearrangements has several advantages. Since the enzyme remains active in mixtures of water and water-soluble organics high concentrations of the substrates can be reached. In contrast, the heme chloroperoxidase from *C. fumago* rapidly denatures in these mixtures. Further, unlike the heme chloroperoxidase from *C. fum*ago,[62] there is no need to control the H_2O_2 concentration.

24.3 Conclusion and Prospects

In many pharmaceuticals, halogens are introduced to increase their activity and halogenated compounds are intermediates in the synthesis of more complex molecules, *e.g.*, the Suzuki-Miyaura palladium catalysed cross-coupling of brominated compounds. In general, the halogenation processes require deleterious reagent, most reaction conditions are corrosive and waste is formed. There is clearly a need for other methods that are more environment-friendly and can be carried out under mild conditions. The research by the Hollmann group on the VCPO illustrates that such a viable enzymatic halogenation method exists and that the enzymatic halogenating methods can be carried out under very mild condtions. The only drawback of the enzymatic halogenation using this class of enzymes is that the halogenation of organic compounds by the hypohalous acids formed will not be regio- or stereoselective. Further the enzyme is not yet commercially available. Nevertheless, the results show that enzymatic halogenation should

make part of the toolbox of organic chemists. Other applications of these VCPOs have also been envisaged based on their stability and on the formation of HOCl and HOBr. Hypohalites have antimicrobial and bleaching properties and patent applications describing the use of VCPO on these subjects are abundant.[64]

References

1. A. M. Held, D. J. Halko and J. K. Hurst, *J. Am. Chem. Soc.*, 1978, **100**, 5732.
2. R. Wever, M. G. M. Tromp, B. E. Krenn, A. Marjani and M. van Tol, *Environ. Sci. Technol.*, 1991, **25**, 446.
3. R. Wever and M. A. van der Horst, *Dalton Trans.*, 2013, **42**, 11778.
4. E. de Boer and R. Wever, *J. Biol. Chem.*, 1988, **263**, 12326.
5. V. Martínez Martínez, G. De Cremer, M. B. J. Roeffaers, M. Sliwa, M. Mukulesh, D. E. De Vos, J. Hofkens and B. F. Sels, *J. Am. Chem. Soc.*, 1988, **130**, 13192.
6. Y. Liu, D. C. O. Thornton, T. S. Bianchi, W. A. Arnold, M. R. Shields, J. Chen and S. A. Yvon-Lewis, *Environ. Sci. Technol.*, 2015, **49**, 3366.
7. P. Coughlin, S. Roberts, C. Rush and A. Willets, *Biotechnol. Lett.*, 1993, **15**, 907.
8. A. Butler and J. N. Carter-Franklin, *Nat. Prod. Rep.*, 2004, **211**, 180.
9. R. Wever, in *Vanadium Biochemical and Molecular Biological Approaches*, ed. H. Michibata, Springer, Dordrecht, Heidelberg, London, New York, ch. 5, 2012, p. 95.
10. R. Wever, B. E. Krenn and R. Renirie, *Methods Enzymol.*, 2018, **605**, 141.
11. R. Wever and P. Barnett, *Chem. – Asian, J.*, 2017, **12**, 1997.
12. S. M. K. McKinnie, Z. D. Miles and B. S. Moore, *Methods Enzymol.*, 2018, **604**, 405.
13. V. Agarwal, Z. D. Miles, J. M. Winter, A. S. Eustaq́uio, A. El Gamal and S. M. Moore, *Chem. Rev.*, 2017, **117**, 5619.
14. J. Latham, E. Brandenburger, S. A. Shepherd, B. R. K. Menon and J. Micklefield, *Chem. Rev.*, 2018, **118**, 232.
15. C. Leblanc, H. Vilter, J. B. Fournier, L. Delage, P. Potin, E. Rebuffet, G. Michel, P. L. Solari, M. C. Feiters and M. Czjzek, *Coord. Chem. Rev.*, 2015, **301–302**, 134.
16. G. Xu and B.-G. Wang, *PLoS One*, 2016, **11**, e0154619.
17. E. G. M. Vollenbroek, L. H. Simons, J. W. P. M. van Schijndel, P. Barnett, M. Balzar, H. L. Dekker, C. van der Linden and R. Wever, *Biochem. Soc. Trans.*, 1995, **23**, 267.
18. P. Barnett, W. Hemrika, H. L. Dekker, A. O. Muijsers, R. Renirie and R. Wever, *J. Biol. Chem.*, 1998, **273**, 23381.
19. A. Messerschmidt and R. Wever, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 392.
20. A. Messerschmidt, L. Prade and R. Wever, *Biol. Chem.*, 1997, **378**, 309.
21. W. Hemrika, R. Renirie, S. Macedo-Ribeiro, A. Messerschmidt and R. Wever, *J. Biol. Chem.*, 1999, **274**, 23820.

22. S. Macedo-Ribeiro, W. Hemrika, R. Renirie, R. Wever and A. Messerschmidt, *J. Biol. Inorg. Chem.*, 1999, **4**, 209.
23. J. W. M. Van Schijndel, P. Barnett, J. Roelse, E. G. M. Vollenbroek and R. Wever, *Eur. J. Biochem.*, 1994, **225**, 151.
24. E. Fernández-Fueyo, M. van Wingerden, R. Renirie, R. Wever, Y. Ni, D. Holtmann and F. Hollmann, *ChemCatChem*, 2015, **7**, 4035.
25. R. Renirie, C. Pierlot, R. Wever and J.-M. Aubry, *J. Mol. Catal. B: Enzym.*, 2009, **56**, 259.
26. N. Tanaka, V. Dumay, Q. Liao, A. J. Lange and R. Wever, *Eur. J. Biochem.*, 2002, **269**, 2162.
27. R. Renirie, C. Pierlot, J.-M. Aubry, A. F. Hartog, H. E. Schoemaker, P. L. Alsters and R. Wever, *Adv. Synth. Catal.*, 2003, **345**, 849.
28. J. W. M. Van Schijndel, E. G. M. Vollenbroek and R. Wever, *Biochim. Biophys. Acta*, 1993, **1161**, 249.
29. N. Tanaka, Z. Hasan and R. Wever, *Inorg. Chim. Acta*, 2003, **356**, 288.
30. Z. Hasan, R. Renirie, R. Kerkman, H. J. Ruijssenaars, A. F. Hartog and R. Wever, *J. Biol. Chem.*, 2006, **281**, 9738.
31. A. But, J. Le Nôtre, E. L. Scott, R. Wever and J. P. M. Sanders, *ChemSusChem*, 2012, **5**, 1199.
32. H. B. Ten Brink, A. Tuynman, H. L. Dekker, W. Hemrika, Y. Izumi, T. Oshiro, H. E. Schoemaker and R. Wever, *Inorg. Chem.*, 1998, **37**, 6780.
33. H. B. Ten Brink, H. E. Schoemaker and R. Wever, *Eur. J. Biochem.*, 2001, **268**, 132.
34. S. Patra, S. Chatterjee, T. K. Si and K. K. Mukherje, *Dalton Trans.*, 2013, **42**, 13425.
35. B. F. Sels, D. E. De Vos and P. A. Jacobs, *J. Am. Chem. Soc.*, 2001, **123**, 8350.
36. M. Ghiacia, M. E. Sedaghata, S. Ranjbaria and A. Gilb, *Appl. Catal., A*, 2010, **384**, 18.
37. V. Conte and B. Floris, *Inorg. Chim. Acta*, 2010, **363**, 1935.
38. A. Podgorsek, M. Zupan and J. Iskra, *Angew. Chem., Int. Ed.*, 2009, **48**, 8424.
39. K. Moriyama, C. Nishinohara, T. Sugiue and H. Togo, *RSC Adv.*, 2015, **5**, 85.
40. L. Getrey, T. Krieg, F. Hollmann, J. Schrader and D. Holtmann, *Green Chem.*, 2014, **16**, 1104.
41. K. M. Manoj and L. P. Hager, *Biochemistry*, 2008, **47**, 2997.
42. R. Vazquez, M. Ayala and F. J. Marquez- Rocha, *Phytochemistry*, 2001, **58**, 929.
43. G. T. Höffler, A. But and F. Hollmann, *Org. Biomol. Chem.*, 2019, DOI: 10.1039/C9OB01884K, in press.
44. M. Ayala, C. V. Batista and R. Vazquez-Duhalt, *J. Biol. Inorg. Chem.*, 2011, **16**, 63.
45. M. A. Pickard and A. Hashimoto, *Can. J. Microbiol.*, 1988, **34**, 998.
46. F. Sabuzi, E. Churakova, P. Galloni, R. Wever, F. Hollmann, B. Floris and V. Conte, *Eur. J. Inorg. Chem.*, 2015, 3519.

47. R. R. Everett, J. R. Kanofsky and A. Butler, *J. Biol. Chem.*, 1990, **265**, 4908.
48. S. H. H. Younes, F. Tieves, D. Lan, Y. Wang, P. Süss, H. Brundiek, R. Wever and F. Hollmann, *ChemSusChem*, 2020, **13**, 97–101.
49. J. Geigert, S. L. Neidleman, D. J. Dalietos and S. K. DeWittt, *Appl. Environ. Microbiol.*, 1983, **45**, 366.
50. N. Itoh, A. K. M. Q. Hasan, Y. Izumi and H. Yamada, *Eur. J. Biochem.*, 1988, **172**, 477.
51. B. E. Krenn, Y. Izumi, H. Yamada and R. Wever, *Biochim. Biophys. Acta*, 1989, **998**, 63.
52. J. J. Dong, E. Fernández-Fueyo, J. Li, Z. Guo, R. Renirie, R. Wever and F. Hollmann, *Chem. Commun.*, 2017, **53**, 6207.
53. I. Z. Munir, S. Hu and J. S. Dordick, *Adv. Synth. Catal.*, 2002, **344**, 1097.
54. N. Carter-Franklin, J. D. Parrish, R. A. Tschirret-Guth, R. D. Little and A. Butler, *J. Am. Chem. Soc.*, 2003, **125**, 3688.
55. J. N. Carter-Franklin and A. Butler, *J. Am. Soc.*, 2004, **126**, 15060.
56. A. Fukuzawa, Y. Takasugi, A. Murai, M. Nakamura and M. Tamura, *Tetrahedron Lett.*, 1992, **33**, 2017.
57. A. Fukuzawa, M. Aye, Y. Takasugi, M. Nakamura, M. Tamura, M. Tamura and A. Murai, *Chem. Lett.*, 1994, **23**, 2307.
58. J. Ishihara, N. Kanoh and A. Murai, *Tetrahedron Lett.*, 1995, **36**, 737.
59. M. Sandy, J. N. Carter-Franklin, J. D. Martiny and A. Butler, *Chem. Commun.*, 2011, **47**, 12086.
60. D. Thiel, F. Blume, C. Jäger and J. Deska, *Eur. J. Org. Chem.*, 2018, 2717.
61. F. van der Pijl, F. L. van Delft and F. P. J. T. Rutjes, *Eur. J. Org. Chem.*, 2015, 4811.
62. D. Thiel, F. Blume, C. Jäger and J. Deska, *Eur. J. Org. Chem.*, 2018, 2717.
63. E. Fernández-Fueyo, S. H. H. Younes, S. van Rootselaar, R. W. M. Aben, R. Renirie, R. Wever, D. Holtmann, F. P. J. T. Rutjes and F. Hollmann, *ACS Catal.*, 2016, **6**, 5904.
64. R. Wever, in *Peroxidases and Catalases: Biochemistry, Biophysics, Biotechnology, and Physiology*, 2nd edn, ed. H. B. Dunford, John Wiley & Sons, Inc., ch. 25, 2010, p. 403.