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Review

Structural and functional comparisons between vanadium haloperoxidase and acid phosphatase enzymes†

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The crystallographic structures of both the vanadium chloroperoxidase and bromoperoxidase enzymes have been determined with either vanadium or phosphate bound at their active site. The amino acids that are involved in phosphate binding in the acid phosphatase enzymes and those that are coordinated to vanadium in the haloperoxidases appear to be conserved between the two classes of enzyme. The detailed active site architecture for enzymes that recognize and use either vanadium or phosphate will be discussed in relation to their proposed enzymatic mechanism. Copyright © 2002 John Wiley & Sons, Ltd.

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INTRODUCTION

Haloperoxidases represent enzymes that are able to catalyse the halogenation of organic compounds in the presence of halide ions and peroxides such as H_2O_2. There are two groups of co-factor-containing haloperoxidase enzymes, those which contain a haem group and those which contain vanadium. There has also been reported a third group which contain no metals, however these enzymes are currently thought to be related to the esterases and lipases because they contain the representative catalytic triad. These enzymes have received increasing attention recently due to their ability to halogenate a range of organic compounds of both commercial and pharmaceutical interest. Several natural products such as halogenated indoles have potent anti-inflammatory and anti-cancer activities (reviewed by Littlechild, 1999).

This article will concentrate on the vanadium-containing enzymes and their relationship to a completely different group of enzymes, the acid phosphatases. A bromoperoxidase enzyme with an absolute requirement for vanadium was first isolated from the brown macro-algae *Ascophyllum nodosum* (Vilter, 1983). One molecule of vanadate per subunit was shown to be required for the enzyme activity. EPR studies (de Boer *et al.*, 1986) and K-edge X-ray absorption studies (Hormes *et al.*, 1988) on the bromoperoxidase from *A. nodosum* showed that the oxidation state of the metal was vanadium V. The redox state of the metal is not thought to change during turnover of the enzyme. It has been proposed that the function of the vanadium is to bind hydrogen peroxide to yield an activated peroxo intermediate, which is able to react with bromide to produce HOBr (Messerschmidt *et al.*, 1997). The natural substrates and the role of the bromoperoxidase in the algae are thought to be involved in the polymerization of polyphenols in order to hold the zygotes to the membrane during the reproductive cycle of the cell (Vreeland *et al.*, 1998). In the case of fungi and lichen suggestions have been made regarding its role in the degradation of plant material or in the organism's defence mechanism due to the biocidal effect of the organohalogens produced (Neidleman and Geigert, 1986; Simons *et al.*, 1995).

The first of the vanadium-containing enzymes to be cloned and sequenced was a chloroperoxidase, (VClPO) from the fungus *Curvularia inaequalis* (Simons *et al.*, 1995). More recently the bromoperoxidase (VBrPO) from the red algae *Corallina pilulifera* and *Cor. officinalis* species has been cloned and expressed in *Escherichia coli* (Shimonishi *et al.*, 1998; Coupe *et al.*, 2001). The carboxyl-terminal 123 residues showed a 45% identity to that of the marine brown algae *A. nodosum* (Vilter, 1995).

The first vanadium-containing enzyme for which a structure became available was the chloroperoxidase from *Cur. inaequalis* (Messerschmidt and Wever, 1996; Messerschmidt *et al.*, 1997). The protein fold of this enzyme is mainly α helical with two four-helix bundles as main structural motifs. The vanadium is bound as hydrogen vanadate (V) in a trigonal bipyrimidal coordination with the bound metal coordinated to three oxygens and a histidine residue of the protein. The multisubunit bromoperoxidase

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Abbreviations used: AVBrPO, *Ascophylum nodosum* vanadium bromoperoxidase; VClPO, vanadium chloroperoxidase; VBrPO, vanadium bromoperoxidase.

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enzyme from *Cor. officinalis* had been crystallized earlier (Rush *et al.*, 1995). The structure of this latter enzyme has now been solved after its crystallization in another more favourable crystal form grown from a high concentration of phosphate (Brindley *et al.*, 1998; Isupov *et al.*, 2000). The chloroperoxidase *Curvularia* enzyme is reported to be a monomer whereas the dodecameric bromoperoxidase enzyme has 12 identical subunits arranged in an unusual 23 symmetrical fashion and not in the 'double doughnut' structure originally proposed from earlier EM studies (Itoh *et al.*, 1986). The dodecamer is arranged with 23 local symmetry about a large central cavity lined with α helices. Each of the cubic faces is made up of a tightly associated dimer, as shown in Plate 1. These dimers are anchored to the central cavity by a loop region, each monomer contributing a single α helix to the lining of the cavity. A dimer of the *Cor. officinalis* enzyme can be superimposed on a monomer of the *Cur. inaequalis* enzyme. The major changes in the secondary structural elements of the enzymes concern the peripheral helices. The two four-helix bundles are present in both enzymes and the long helices that pass across the two-fold axis in the *Cor. officinalis* enzyme are extended in the *Cur. inaequalis* enzyme. The fungal enzyme only contains a single vanadium-binding site and this is partially covered by a movement of a loop region. The second vanadate site has been lost and the region where it should occur is now on the surface of the enzyme.

COR. OFFICINALIS VBrPO

The *Cor. officinalis* VBrPO subunit measures approximately $85 \times 56 \times 55$ Å in size. It is folded into a single α–β type domain of 595 amino acids. The first 10 N-terminal amino acids point away from the body of the domain that is composed of 19 α-helices from six to 26 amino acids in length. There are also eight 3_{10}-helices and 14 β-strands. The β-strands are mainly involved in β-hairpins. One of the surfaces of the subunit is flat.

The *Cor. officinalis* VBrPO dimer structure is shown in Plate 1. The flat surfaces of the two subunits complement each other covering an area of 5260 Å^2 or 20.5% of the subunit solvent accessible surface. The residues 123–124 from one subunit and 368–369 from the other subunit in the dimer form a parallel two-stranded β-sheet. The β-hairpins 270–273 and 276–279 from each subunit in the dimer form an antiparallel four-stranded β-sheet. The vanadate binding site is located at the bottom of the active site cleft which is about 20 Å deep and 14 Å wide. The active site cleft is formed from residues of two different subunits in the dimer.

The *Cor. officinalis* VBrPO dodecamer measures ~150 Å in diameter. Twelve subunits are arranged with 23 cubic point group symmetry (Plate 2). This symmetry has only been observed in two other enzyme structures to date—a DNA protection protein Dps from *E. coli* (Grant *et al.*, 1998) and an ornithine carbamoyltransferase from *Pyrococcus furiosus* (Villeret *et al.*, 1998). Each of the cubic faces is made up of a dimer. The N-terminal region of each subunit contributes to the formation of the central cavity. The diameter of this cavity is about 26 Å and it does not have any specific charge or hydrophobic properties. This feature is therefore unlikely to bind metals and is proposed to have a structural role.

An additional 3245 Å^2 of the solvent accessible area of each subunit is buried upon dodecamer formation from dimers. This gives an overall figure of 8505 Å^2 for the buried area for each subunit which amounts to 33.2% of its surface. Each subunit makes at least one H-bond with nine other subunits within the dodecamer.

The vanadium-binding site in the VBrPO is located on the bottom of a deep cavity formed by residues from both subunits. The residues of one subunit form the bottom of the active site cleft and the top of the cleft is formed predominantly from the residues of the other subunit of the dimer. The involvement of residues from the neighbouring subunit in the active site cleft would suggest that the VBrPO dimer is required to maintain specificity and stereoselectivity for halogenation of organic substrates, despite the fact that all vanadate binding residues are coming from one subunit.

The bottom of the cleft is formed by the residues of helices α10, α11, α14, α17, α18 and α19 of one subunit. The top of the cleft is formed by the residues of helix α9 and some loops from the same subunit and helices α11, α12 and α13, the β-hairpin (251–253 and 256–258) and the divalent cation binding motif of the neighbouring subunit. The long helix α11 contributes its residues to the active site clefts of both subunits.

In the VBrPO structure initially solved the active site is occupied by inorganic phosphate. The phosphate is located at the N-terminus of the helix α17 at the bottom of the cleft and is stabilized by its dipole moment and H-bonds with Ser483 OG, Gly484 N and His485 ND1. The His551 which is proposed to make a covalent bond to the vanadium is H-bonded to one of the phosphate oxygens. Additionally the phosphate forms salt bridges with Lys398 (helix α13), Arg406 (helix α14) and Arg545 (helix α18).

There are several hydrophobic patches and charged residues which could provide binding sites for the organic substrates. Attempts to co-crystallize VrBPO with various known organic substrates or halogen ions or to obtain the complexes by soaking of the crystals are in progress at Exeter. Divalent cations thought to be Mg^{2+} or Ca^{2+} bind close to the subunit interface in the dimer on the top of the active site cleft. The metal is coordinated by main chain oxygens of Phe359, Gln361 and Gln368 and carboxyl groups of Asp363 and Asp366. The distance between the Mg^{2+} ion of one subunit of the dimer and the phosphorus of the inorganic phosphate bound at the active site of the other subunit is 19 Å. Divalent cations seem to be necessary to maintain the structure of the active site cleft and for dimer interaction.

COMPARISON WITH OTHER VANADIUM HALOPEROXIDASES

The structure of the chloroperoxidase from fungus *Cur. inaequalis* (Messerschmidt and Wever, 1996) which is a two domain protein can be superimposed with the VrBPO dimer. Many α-helices of each chloroperoxidase domain are structurally equivalent to some α-helices in the two different

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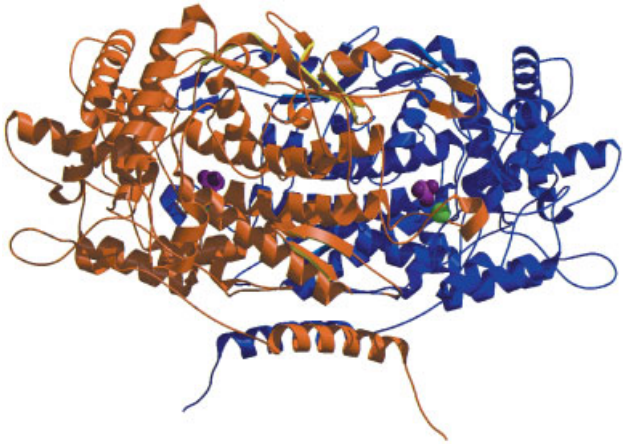

Plate 1. A ribbon diagram representation of the *Corallina* VBrPO dimer viewed normal to the molecular dyad. The inorganic phosphate found in the active site is shown in CPK space-filling mode. The figure was created using BOBSCRIPT (Esnouf, 1999) and rendered with Raster3D (Merritt and Bacon, 1997).

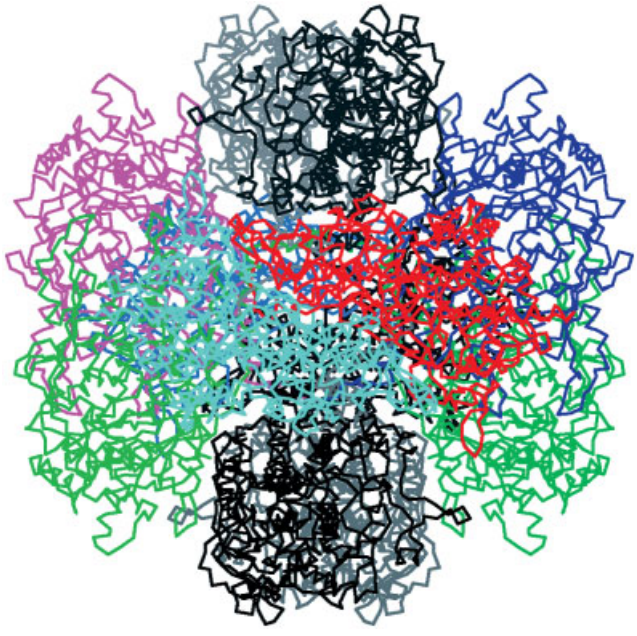

Plate 2. A diagram showing the Cα backbone trace of the *Corallina* VBrPO dodecamer viewed along the molecular dyad. The identical individual subunits are shown in different colours for display purposes.

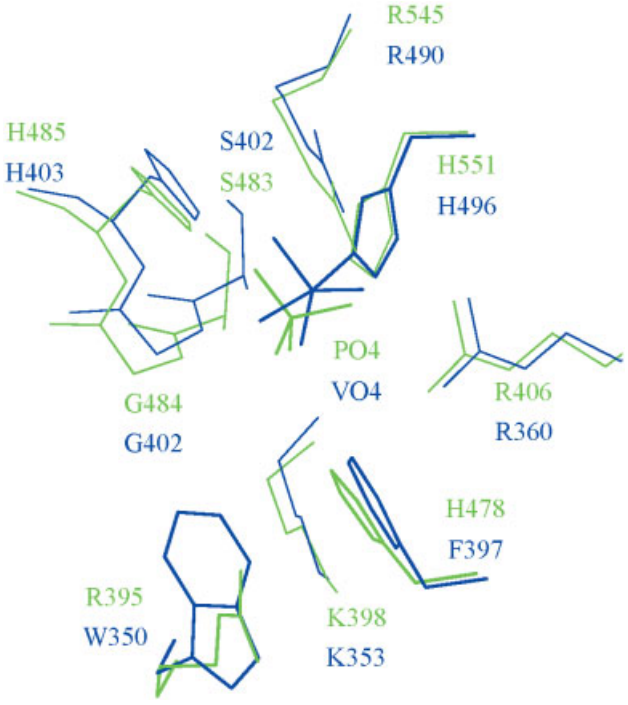

Plate 3. A superposition of the active sites of the *Corallina* VBrPO enzyme (shown in green) and the holo vanadium chloroperoxidase from *Cur. inaequalis* (Messerschmidt and Wever, 1996; pdb code 1VNI shown in blue). The superposition was made and refined using the program O (Jones *et al.*, 1991). In the centre of the picture is shown vanadate, which makes a covalent bond to NE2 of His496 in the chloroperoxidase and inorganic phosphate bound at the vanadate site in *Corallina* VBrPO. Side chains for residues implicated in the catalytic mechanism are shown as bonds and labeled in the respective colour. The conserved residues or those with similar locations are shown.

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VBrPO subunits. This suggests a divergent evolutional relationship between the VBrPO and the chloroperoxidase. It would appear that the VBrPO represents an ancestral enzyme with a two-subunit catalytic structure while the chloroperoxidase has undergone gene duplication as suggested from its X-ray structure.

The VBrPO dimer and chloroperoxidase monomer can be superimposed with a rmsd of 1.9 Å for 259 matching Cα atoms. The chloroperoxidase has lost the catalytic function of its N-terminal domain. It would appear that the chloroperoxidase C-terminal domain has retained structure and residues related to catalytic functions and has lost most of the features related to a second active site during evolution. On the contrary the N-terminal domain retained mainly features which contribute to the active site cleft in the catalytic domain and lost features of its own active site. However the number of residues per catalytic site has remained similar (595 in VBrPO and 609 in the chloroperoxidase).

Since the structure of the VBrPO was solved a related enzyme from *A. nodosum* (AVBrPO) also became available (Weyland *et al.*, 1999). AVBrPO and VBrPO enzymes have 33% amino acid sequence identity. The long α-helices that make up the core of the molecule seem to be conserved between the two enzymes; many small helices and β-strands which are on the surface differ between the two structures. The number and location of the β-strands is also different between the two structures. The divalent cations do not occur in the AVBrPO structure and indeed the α-helix/β-strand cation binding feature of VBrPO is also not observed in the *A. nodosum* enzyme which has a three-residue deletion in this region.

Both AVBrPO and VBrPO show high thermostability as well as stability to organic solvents and to high concentrations of hydrogen peroxide. The thermostability of these enzymes, which originate from mesophilic organisms, is unexpected and could be due to adaptation to harsh environmental conditions of overheating and dehydration which the algae endure at low tide. It seems that the two enzymes have developed different mechanisms of stability. In the dimeric AVBrPO enzyme all of the cysteine residues are involved in three intersubunit and one intrasubunit disulphide bonds which make a significant contribution to the enzyme stability. None of these cysteines is conserved in VBrPO. The only two cysteines in VBrPO are located far apart and are observed in the reduced form. The stability of VBrPO seems to result from the high dodecameric oligomerization that buries an extra 12% of the subunit solvent accessible area and from the binding of the divalent cations.

All of the residues involved in the vanadate binding are conserved between the two algal bromoperoxidases and the vanadium chloroperoxidase from the fungus *Cur. inaequalis*. Only five residues out of 17 lining the wall of the active site cavity and not involved in the vanadate binding are conserved between VBrPO and AVBrPO. None of these five residues is structurally conserved in the *Cur. inaequalis* chloroperoxidase structure. His478 is located at the position of Phe397, which was suggested to bind the chloride in the chloroperoxidase active site (Messerschmidt and Wever, 1996). This substitution and H-bonding of Asp333 (Asp278) to the catalytic His485 (His418) were suggested to be the reasons for a preference for bromination rather than chlorination for AVBrPO (Weyand *et al.*, 1999) and indeed His478 and Asp333 are conserved in all VBrPOs. An active site serine was suggested as a possible candidate to form a carbon-bromine covalent bond from EXAFS studies (Dau *et al.*, 1999). However the solvent accessibility of this Ser483 in VBrPO (Ser416 in AVBrPO) seems to be low in the phosphate-bound form of the enzyme. The significant structural changes within the active site during the catalytic cycle which would make it accessible have been ruled out for the chloroperoxidase enzyme on the basis of structural studies on mutant proteins (Macedo-Ribeiro *et al.*, 1999).

Only three hydrophilic residues and no charged residues except those involved in the vanadate binding are observed within 7.5 Å from the vanadate O^4 oxygen in the *A. nodosum* structure (Weyand *et al.*, 1999). There are three charged residues within approximately the same sphere in *Cor. officinalis* VBrPO. These residues could affect the substrate specificity and stereoselectivity of the reaction.

Recent studies on the brown algae *Fucus distichus* have revealed that its bromoperoxidase is arranged as a dodecamer similar to that found in the red algae (Vreeland *et al.*, 2000).

SITE-DIRECTED MUTAGENESIS

Vanadium haloperoxidases have some amino acids in common in the active site (Plate 3) and subtle differences that allow them to distinguish their specific cofactor (Br^-, Cl^-, I^-). Superposition of these active sites allowed the identification of the definite differences and led to the application of site-directed mutagenesis studies on *Cur. inaequalis* (Hemrika *et al.*, 1999). Hemrika's work demonstrated the importance of the histidine (His496) in holding the vanadate in the active site and the decrease in activity when the aminoacids Arg360, Arg490 and Lys353 were mutated. Transformation to bromoperoxidase activity was achieved but not up to the activity found in native bromoperoxidases.

ACID PHOSPHATASES

Interestingly the enzyme active sites of the vanadium haloperoxidases have been shown to have some conservation in a completely different group of enzymes, the acid phosphatases (Hemrika *et al.*, 1997; Neuwald, 1997). It appears that nature has developed the same binding site for vanadium and phosphate. The acid phosphatases are classified into several different groups. Sequence motifs are shared between the vanadium-dependent chloroperoxidase and membrane-associated phosphatases including type 2 phosphatidic acid phosphatase, bacterial acid phosphatases, mammalian glucose-6-phosphatases and the *Drosophila* developmental protein wunen. This similarity in active sites has been confirmed by the structural determination of an acid phosphatase from *Escherichia blattae* (Ishikawa *et al.*, 2000). The same binding motif for phosphate and vanadate supports the proposal that the vanadium (V) passes through a tetrahedral intermediate during the reaction mechanism (see below). The alignment

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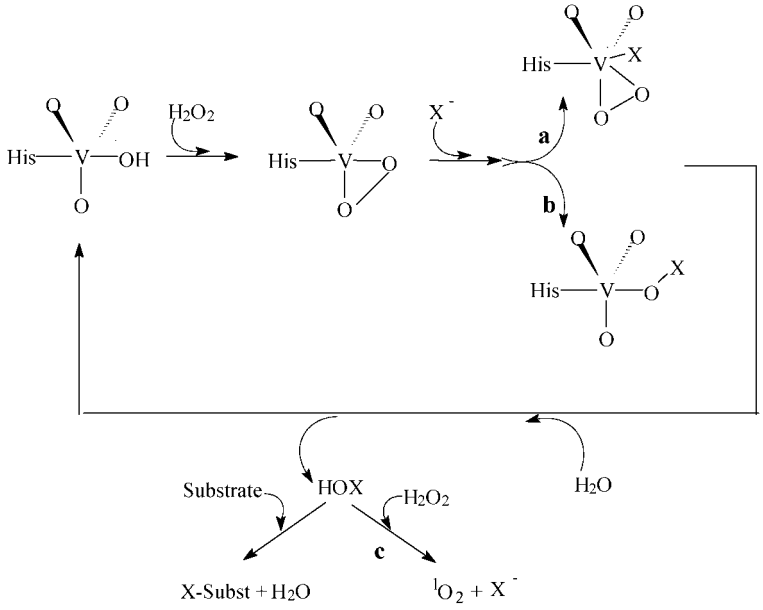

Figure 1. Diagrammatic representation of the proposed mechanism a, b and c for the vanadium haloperoxidase enzymes.

of several vanadium bromoperoxidase/chloroperoxidase enzymes with acid phosphatases highlighting the conserved active site residues is shown in Plate 4. Haloperoxidases have also been reported to be able to carry out phosphatase reactions by substituting the vanadate group with phosphate (Hemrika *et al.*, 1997; Renirie *et al.*, 2000), which further supports the connection. The structural relationship with the family of acid phosphatases could lead to attempts to simulate and even improve the phosphatase activity of the related enzymes (Wever *et al.*, 2001). Recently Wever and collaborators have shown that vanadium substituted bacterial acid phosphatases from *Shigella* and *Salmonella* can function as bromoperoxidases, although much less efficiently than the vanadium bromide enzyme from *A. nodosum* (Tanaka *et al.*, 2002). In addition a novel variant of bacterial acid phosphatases has recently been studied structurally by molecular modelling using the structure of the *E. blattae* acid phosphatase as a model (Madan Babu *et al.*, 2002). This enzyme is a virulence-associated ATP diphosphohydrolase called apyrase that is found in the periplasm of *Shigella*, which has a 50% sequence identity to the *E. blattae* enzyme. The apyrase however has negligible phosphatase activity despite the active site residues being conserved between the two enzymes. It does however hydrolyse ATP at a pH optimum of 7.0 and is therefore considered to be an exo-pyrophosphatase rather than a phosphatase (Madan Babu *et al.*, 2002). It is proposed that the apyrase is central to *Shigella's* pathogenesis and could be involved in actin polymerization.

CATALYTIC MECHANISM OF VANADIUM BROMOPEROXIDASES

In 1987, experiments with different substrates suggested that the bromination reaction did not involve the formation of molecular bromine (Itoh *et al.*, 1987). The formation of enol intermediates was also proposed (Everett and Butler, 1987).

An extensive steady-state kinetics study on *A. nodosum* bromoperoxidase gave the first ideas on possible mechanisms (de Boer and Wever, 1988). The vanadate was found to not change its oxidation state during catalysis (Arber *et al.*, 1989). The formation of HOBr and HOCl species was already known in other types of peroxidases and it is now a widely accepted part of the mechanism.

Several mimetic systems were synthesized in order to establish the different steps of the mechanism. Vanadium-peroxo complexes proved to be able to catalyse bromination reactions (Costa Pessoa *et al.*, 1994; Colpas *et al.*, 1996; Totaro *et al.*, 2000) and the involvement of histidine in a vanadate complex was modelled successfully in a synthetic compound (Fritzsche *et al.*, 1993).

The first step is therefore clearly established and the structure of the peroxo intermediate has been confirmed by X-ray diffraction experiments in chloroperoxidase (Messerschmidt *et al.*, 1997) and by ^{17}O NMR spectroscopy with bromoperoxidase (Casný *et al.*, 2000). The crystal structures of the native and peroxidase forms of chloroperoxidase from the fungus *Cur. inaequalis* have shown the vanadium coordinated by four non-protein oxygen atoms and one nitrogen from histidine in a trigonal bipyrimidal fashion. A peroxide form of the enzyme shows peroxide bound to the vanadium in an η^2-fashion after release of the apical oxygen ligand. It is known that the next stage of the mechanism involves halide binding to the peroxo complex and that it ends with release of HOX (X = halide). The structure of the halide-peroxo-vanadium complex remains unclear. The mechanism that is more widely accepted can be seen in Fig. 1.

Two alternative forms of halide binding have been proposed, illustrated in Fig. 1(a) (Messerschmidt *et al.*,

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Plate 4. (**above**). A sequence alignment of the known vanadium chloroperoxidase and bromoperoxidase enzymes and the acid phosphatases that show similarity in their active site region. The conserved catalytic residues common to the two groups of enzyme are highlighted in yellow. The alignment was carried out using the program BLASTP 2.2.3 (Altschul *et al.*, 1997). The abbreviated code for the enzymes included in the alignment is as follows, *Cor. pi1*, vanadium bromoperoxidase 1 (*Corallina pilulifera*); *Cor. of.*, vanadium bromoperoxidase (*Corallina officinalis*); *Cor. pi2*, vanadium bromoperoxidase 2 (*Corallina pilulifera*); *Asc. no.*, vanadium bromoperoxidase (*Ascophyllum nodosum*); *Fuc. di.*, vanadium bromoperoxidase (*Fucus distichus*); *Dre. bi.*, vanadium chloroperoxidase (*Drechslera biseptata*); *Cur. in.*, vanadium chloroperoxidase (*Curvularia inaequalis*); *Emb. di.*, vanadium chloroperoxidase (*Embellisia didymospora*); *Dei. ra.*, vanadium chloroperoxidase-related protein (*Deinococcus radiodurans*); *Esc. bl.*, acid phosphatase (*Escherichia blattae*); *Ent. ae.*, acid phosphatase (*Enterobacter aerogenes*); *Rao. pl.*, acid phosphatase (*Raoultella planticola*); *Mor. mo.*, acid phosphatase (*Morganella morganii*); *Shi. fl.*, PhoN1, periplasmic non specific acid phosphatase (*Shigella flexneri*); *Pro. st.*, PhoN acid phosphatase (*Providencia stuartii*); *Pre. in.*, acid phosphatase (PiACP) (*Prevotella intermedia*); *Zym. mo.*, acid phosphatase (*Zymomonas mobilis*); *Sal. ty.*, acid phosphatase (*Salmonella typhimurium*); *Cau. cr.*, acid phosphatase (*Caulobacter crescentus CB15*).

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Plate 5. Superimposition of the *Cor. officinalis* vanadium haloperoxidase enzyme (Isupov *et al.*, 2000; PDB code 1QHB) and the acid phosphatase from *E. blattae* (Ishikawa *et al.*, 2000; PDB code 1EOI). The structures were superimposed using the program O (Jones *et al.*, 1991), displayed using the program BOBSCRIPT (Esnouf, 1999) and rendered with Raster3D (Merritt and Bacon, 1997). The bound phosphate and Mg^{2+} of the vanadium haloperoxidase are shown in space filling mode.

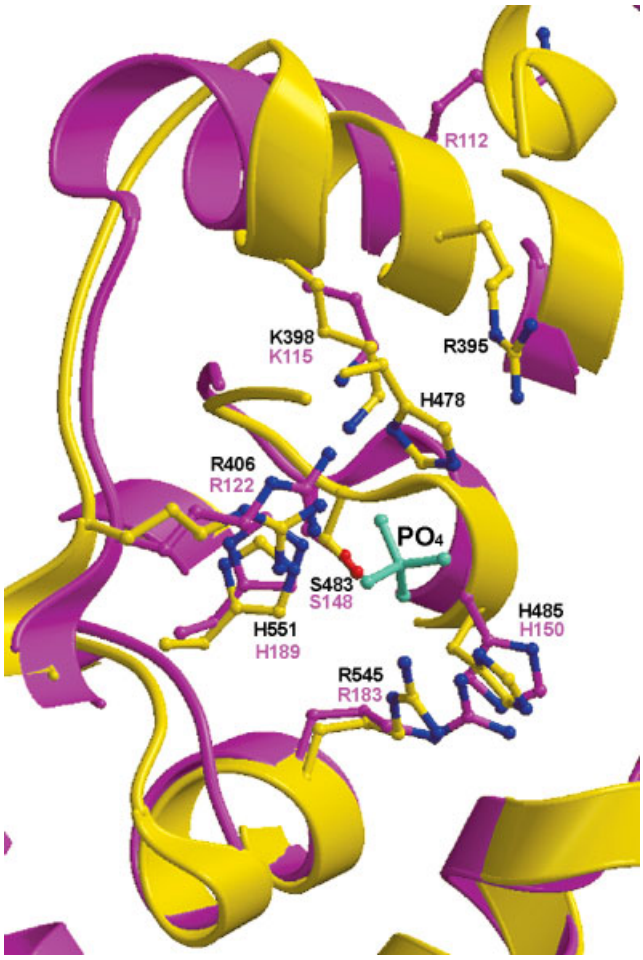

Plate 6. (**left**). A superimposition of the active site region of *Cor. officinalis* vanadium haloperoxidase enzyme (yellow) and the acid phosphatase from *E. blattae* (magenta) (prepared as described in Plate 5), showing the amino acid side chains involved or close to the bound phosphate (cyan).

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1997) and Fig. 1(b) (Weyand *et al.*, 1999). Kinetic studies have shown the release of singlet oxygen (Everett *et al.*, 1990), indicating a secondary reaction as seen in Fig. 1(c). Recently, EXAFS studies have discarded option (a), but these results point towards the binding of the halogen to an aromatic residue, which is in disagreement with the known chemical data (Dau *et al.*, 1999; Rehder *et al.*, 2000). Several experiments have been performed in order to establish the influence of the halide cofactor and hydrogen peroxide concentration in the reaction (de Boer *et al.*, 1988; Everett *et al.*, 1990; Soedjak *et al.*, 1995). High concentrations of halide or hydrogen peroxide result in inhibition of the activity of the enzyme, but this inhibition is reversible except at very low pH conditions. However, this mechanism does not explain the regioselectivity displayed by the enzyme in the reaction with certain substrates. It is necessary to determine where the HOX interacts in order to perform a substrate halogenation. EXAFS studies demonstrated that the amino acids that constitute the active site do not undergo any change in the presence or absence of halide (Arber *et al.*, 1989). No substrate-binding site is known either, but the specificity of the halogenation positions suggests that some type of interaction should hold the substrate in an adequate orientation. A review on vanadium haloperoxidases (Butler, 1999) discusses their mechanism and the construction of functional mimics of the enzyme.

The acid phosphatases that are related to the vanadium haloperoxidase enzymes are considered to be histidine phosphatases and as such would use the histidine for nucleophilic attack in the first step of the proposed reaction (Ishikawa *et al.*, 2000). The acid phosphatase of *E. blattae* is the only bacterial acid phosphatase which structure has been determined to date. Although the overall sequence homology is low in comparison to the vanadium bromoperoxidases, 21.7, 17.8 and 15.8% identity to the *Cor. officinalis, A. nodosum* and *Cur. inaequalis* enzymes, respectively, the structural similarity is apparent, as shown in Plate 5. The amino acids that constitute the active site, mainly those involved in the direct bonding of the phosphate/vanadate, are conserved, as shown in Plate 6.

In the *E. blattae* acid phosphatase enzyme a large conformational change is observed in the side chain of His150, the putative catalytic residue, which flips over towards molybdate to form a hydrogen bond with the metal oxyanion. This change is not observed in apo and holo vanadium chloroperoxidase structures (Messerschmidt *et al.*, 1997) and is not seen in the phosphate bound form of the *Cor. officinalis* structure described here.

Studies on the vanadium haloperoxidase enzymes described here provide some insight into the structural properties of the class of histidine acid phosphatase enzymes which in many cases are membrane-bound and difficult to crystallize. The structural and mechanistic comparisons that can be made provide insight into the evolution of these two apparently different enzymes and how they have developed to function as either acid phosphatases or haloperoxidases in fungi and algal species.

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