

# Modeling the Catalytic Site of Vanadium Bromoperoxidase: Synthesis and Structural Characterization of Intramolecularly H-bonded Vanadium(V) Oxoperoxo Complexes, $[\text{VO}(\text{O}_2)(^{\text{NH}_2}\text{pyg}_2)]\text{K}$ and $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]\text{K}$

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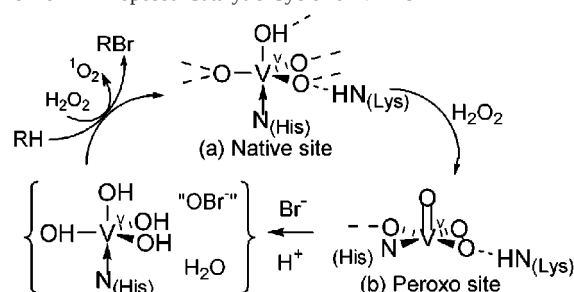
Vanadium haloperoxidases (VHPO) catalyze the peroxidative halogenation of organic substrates. Crystallographic studies suggest that hydrogen bonding from a lysine side chain to the vanadium(V)-bound peroxo group may facilitate oxidation of halides ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ). A ligand with pendant  $\text{NH}_2$  functionality, *N*-(2-pyridylmethyl-6-amino) iminodiacetic acid ( $\text{H}_2^{\text{NH}_2}\text{pyg}_2 \cdot 2\text{HCl}$ ) has been designed to explore the effects that H-bonding from Lys may have on peroxide activation. The first structural characterization of VBrPO model complexes  $[\text{VO}(\text{O}_2)(^{\text{NH}_2}\text{pyg}_2)]\text{K}$  and  $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]\text{K}$  which demonstrate direct intramolecular H-bonding between an amine functionality and V(V)-bound peroxide is reported. The distances between  $\text{NH}_2$  proton and bound peroxo moiety  $\{d(\text{N}(1)-\text{H}\cdots\text{O})$ : 2.637(4) Å in  $[\text{VO}(\text{O}_2)(^{\text{NH}_2}\text{pyg}_2)]\text{K}$ , and 2.640(8) and 2.6919(8) Å in  $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]\text{K}$  are indicative of intramolecular H-bonding. The intramolecular H-bond strength in  $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]^-$  is estimated at 6 kcal/mol by  $^1\text{H}$  NMR studies and demonstrates that the H-bond interaction is also significant in solution.

Vanadium haloperoxidases (VHPO), found in marine algae, lichens, and certain terrestrial fungi, catalyze the halogenation (Cl, Br, I) of organic substrates or the halide-assisted disproportionation of hydrogen peroxide (Scheme 1).<sup>1</sup> The X-ray structures of vanadium chloroperoxidase from *Curvularia inaequalis*<sup>2</sup> and the vanadium bromoperoxidases from *Corallina officinalis*<sup>3</sup> and *Ascophyllum nodosum*<sup>4</sup> reveal that the vanadium active site resembles vanadate ( $\text{HVO}_4^{2-}$ ) that is coordinated to the protein by one histidine residue in a trigonal bipyramidal geometry (structure a, Scheme 1). The histidine which directly binds V(V) and the amino acids involved in H-bonding to the vanadate oxygen atoms are

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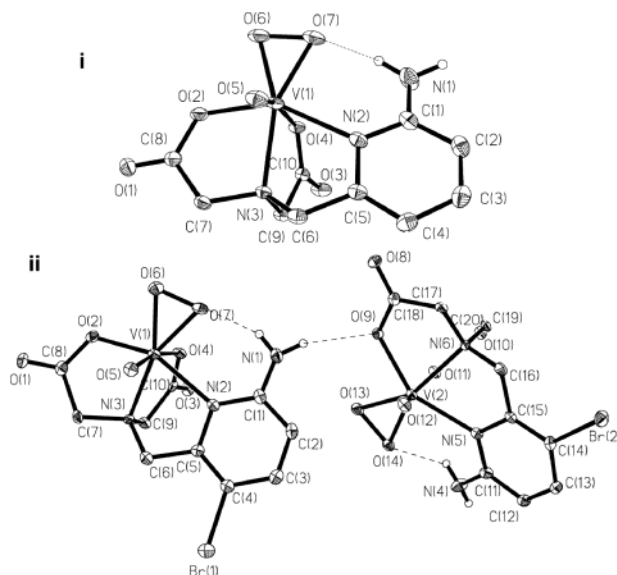
**Scheme 1.** Proposed Catalytic Cycle for VHPO



conserved. Structural characterization of vanadium chloroperoxidase (VCIPO) from *C. inaequalis* in the presence of  $\text{H}_2\text{O}_2$  demonstrates that vanadium(V) is coordinated axially by a terminal oxo group, and equatorially by peroxide, histidine, and an oxide ligand, in a square pyramidal geometry (structure b, Scheme 1).<sup>5</sup> In light of the importance of H-bonding in the regulation of metal ion reactivity,<sup>6</sup> a striking feature at the active site of VCIPO is the apparent 2.76 Å H-bond between Lys<sub>353</sub> and the bound peroxide. Although peroxo derivatives of vanadium bromoperoxidases (VBrPO) have not yet been structurally characterized, the conserved amino acid residues found at the active sites of V-BrPO's (*C. officinalis* and *A. nodosum*) suggest that the redundant Lys residues (Lys<sub>398</sub>, Lys<sub>349</sub>, respectively) are also in positions to H-bond to a V(V)–peroxo moiety in VBrPO's.

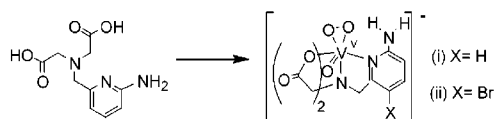
Mechanistic studies of VHPO and VHPO model complexes suggest that peroxide activation may be achieved by

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- (5) Messerschmidt, A.; Prade, L.; Wever, R. *Biol. Chem.*, **1997** *378*, 309. (2.24 Å resolution.)
- (6) In particular its effect on the heterolytic cleavage of O–O bonds in heme enzymes: peroxidase and catalase. Poulos, T. L.; Kraut, J. *J. Biol. Chem.* **1980**, *255*, 8199–8205.



**Figure 1.** ORTEP views of  $[\text{VO}(\text{O}_2)(\text{NH}_2\text{-pyg}_2)]^-$  (i) and  $[\text{VO}(\text{O}_2)(\text{BrNH}_2\text{-pyg}_2)]^-$  (ii). Only  $\text{NH}_2$  protons are shown for clarity. Selected bond lengths ( $\text{\AA}$ ) and angles (deg) for i:  $\text{V}-\text{O}(5)$  1.608(2),  $\text{V}-\text{O}(6)$  1.859(2),  $\text{V}-\text{O}(7)$  1.880(2),  $\text{O}(6)-\text{O}(7)$  1.419(2),  $\text{N}(1)-\text{H}\cdots\text{O}(7)$  2.637(4);  $\text{O}(5)-\text{V}(1)-\text{N}(2)$  93.54(7),  $\text{O}(5)-\text{V}(1)-\text{O}(4)$  166.30(7),  $\text{O}(5)-\text{V}(1)-\text{O}(7)$  102.13(7),  $\text{O}(5)-\text{V}(1)-\text{O}(6)$  104.16(7),  $\text{O}(6)-\text{V}(1)-\text{O}(7)$  44.61(8),  $\text{N}(1)-\text{H}-\text{O}(7)$  144(3). For complex 1 of ii:<sup>14</sup>  $\text{V}(1)-\text{O}(5)$  1.605(4),  $\text{V}(1)-\text{O}(6)$  1.868(4),  $\text{V}(1)-\text{O}(7)$  1.883(4),  $\text{O}(6)-\text{O}(7)$  1.429(6),  $\text{N}(1)-\text{H}\cdots\text{O}(7)$  2.640(8),  $\text{N}(1)-\text{H}\cdots\text{O}(9)$  3.16;  $\text{O}(5)-\text{V}(1)-\text{N}(2)$  91.5(2),  $\text{O}(5)-\text{V}(1)-\text{O}(4)$  165.1(2),  $\text{O}(5)-\text{V}(1)-\text{O}(7)$  103.1(2),  $\text{O}(5)-\text{V}(1)-\text{O}(6)$  104.9(2),  $\text{O}(6)-\text{V}(1)-\text{O}(7)$  44.8(2),  $\text{N}(1)-\text{H}\cdots\text{O}(7)$  125(4),  $\text{N}(1)-\text{H}\cdots\text{O}(9)$  156.

#### Scheme 2<sup>a</sup>



<sup>a</sup> (i)  $\text{KVO}_3$ ,  $\text{H}_2\text{O}_2$ ; (ii)  $\text{KVO}_3$ ,  $\text{H}_2\text{O}_2$ ,  $\text{KBr}$ .

protonation of the  $\text{V}(\text{V})$ -bound peroxo group to generate a side-on bound hydroperoxide complex.<sup>5</sup> It has been proposed that an increase in positive charge on  $\text{O}_{\text{peroxo}}$ , by protonation, makes attack by halide more favorable.<sup>7,8</sup> To explore the effects that H-bonding from Lys may have on peroxide activation, we have designed the ligand  $N$ -(2-pyridylmethyl-6-amino)iminodiacetic acid, ( $\text{H}_2\text{NH}_2\text{-pyg}_2$ ), with pendant  $\text{NH}_2$  functionality.<sup>9</sup> We report the first structural characterization of  $\text{VBrPO}$  model complexes which demonstrate significant intramolecular H-bonding between a pendant amine functionality and  $\text{V}(\text{V})$ -bound peroxide (Figure 1).<sup>10</sup>

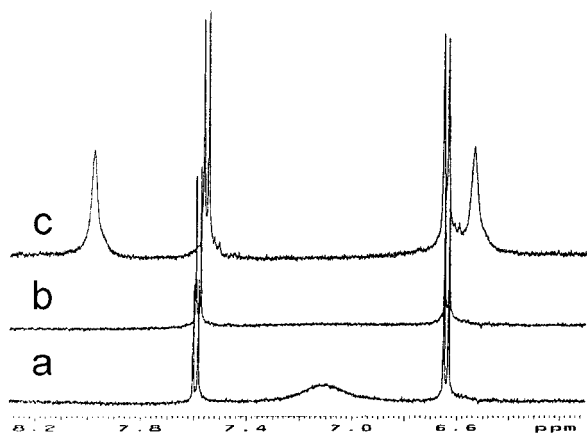
$[\text{VO}(\text{O}_2)(\text{NH}_2\text{-pyg}_2)]\text{K}$  is obtained by adding  $\text{H}_2\text{NH}_2\text{-pyg}_2 \cdot 2\text{HCl}$  to an aqueous slurry of  $\text{KVO}_3$ , followed by addition of  $\text{H}_2\text{O}_2$  at pH 4 (Scheme 2i). In the presence of  $\text{Br}^-$  a bromoperoxidase type reaction occurs, giving  $[\text{VO}(\text{O}_2)(\text{BrNH}_2\text{-pyg}_2)]\text{K}$ ,

in which the pyridine ring is brominated para to the amine moiety (Scheme 2ii).<sup>11,12</sup> The molecular structures of  $[\text{VO}(\text{O}_2)(\text{NH}_2\text{-pyg}_2)]\text{K}$ <sup>13</sup> and  $[\text{VO}(\text{O}_2)(\text{BrNH}_2\text{-pyg}_2)]\text{K}$  resemble those of other  $\text{V}(\text{V})$ -peroxo complexes which are coordinated by tripodal, tetradentate amine ligands.<sup>7</sup> Both complexes have pentagonal bipyramidal structures with axial carboxylate  $\text{O}(4)$  and terminal oxo ligands. Peroxide binds symmetrically in the equatorial plane in a side-on fashion, with bond lengths typical for  $\text{V}(\text{V})$ -oxo-monoperoxo complexes ( $d(\text{V}-\text{O}_{\text{peroxo}})$  ca. 1.87  $\text{\AA}$ ).<sup>15</sup> The distances between the amine nitrogen and bound peroxo moiety are indicative of intramolecular H-bonding:  $d(\text{N}(1)-\text{H}\cdots\text{O}(7)) = 2.637(4)$   $\text{\AA}$  in  $[\text{VO}(\text{O}_2)(\text{NH}_2\text{-pyg}_2)]\text{K}$ ; and  $d(\text{N}(1)-\text{H}\cdots\text{O}(7)) = 2.640(8)$ ,  $d(\text{N}(1)-\text{H}\cdots\text{O}(14)) = 2.62$   $\text{\AA}$  in  $[\text{VO}(\text{O}_2)(\text{BrNH}_2\text{-pyg}_2)]\text{K}$ . It is noteworthy that the  $\text{LysN}_{353}-\text{O}(\text{peroxo})$  bond length of 2.67  $\text{\AA}$  (*C. inaequalis*) is nearly the same.

To our knowledge  $[\text{VO}(\text{O}_2)(\text{NH}_2\text{-pyg}_2)]\text{K}$  and  $[\text{VO}(\text{O}_2)(\text{BrNH}_2\text{-pyg}_2)]\text{K}$  are the first models of  $\text{VHPO}$ 's in which a direct intramolecular H-bond bridges an amine proton and a vanadium-peroxo moiety.<sup>16</sup> Variable-temperature  $^1\text{H}$  NMR was used to investigate the integrity of the H-bonding (Figure 2).<sup>17</sup> At room temperature the  $\text{NH}_2$  protons in both complexes appear as a broad singlet, indicating that the protons are in rapid exchange between the two possible environments exemplified by their solid-state structures. At lower temperatures the proton resonances diverge into two sharper singlets, one moving upfield and the other downfield, indicating that the latter proton is deshielded as a result of its interaction with the peroxo oxygen  $\text{O}(7)$ .<sup>18</sup> In the case of  $[\text{VO}(\text{O}_2)(\text{BrNH}_2\text{-pyg}_2)]\text{K}$  the  $\text{NH}_2$  protons (6.5 and 8.0 ppm at 233 K) coalesce at ca. 283 K (500 MHz), corresponding to a  $\Delta G^\ddagger$  value of 12.4 kcal/mol for rotation about the C-N bond. If

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- (8) Butler, A.; Baldwin, A. H. *Struct. Bonding* **1997**, *89*, 109.
- (9) Synthesis of  $\text{H}_2\text{NH}_2\text{-pyg}_2 \cdot 2\text{HCl}$  is achieved via reaction of  $N$ -[6-(bromomethyl)-2-pyridinyl]-2,2-dimethylpropanamide with iminodiacetic acid disodium salt and is similar to that employed by Martell et al. for synthesis of  $N$ -(*o*-hydroxybenzyl)iminodiacetic acid. Harris, W. R.; Motekaitis, R. J.; Martell, A. E. *Inorg. Chem.* **1975**, *14*, 974.
- (10) Two nearly identical, but crystallographically distinct, molecules of  $[\text{VO}(\text{O}_2)(\text{BrNH}_2\text{-pyg}_2)]\text{K}$  were found and exhibit intermolecular H-bonding, as shown in Figure 1ii.

- (11) Bromination of the ligand may be effected by  $[\text{VO}(\text{O}_2)(\text{NH}_2\text{-pyg}_2)]\text{K}$ , or it could also occur via oxidation of  $\text{Br}^-$  by  $\text{H}_2\text{O}_2$  under the acidic conditions employed for synthesis.
- (12) Similar ligand halogenation has been observed by Thiel et al.: Glas, H.; Herdtweck, E.; Artus, G. R. J.; Thiel, W. R. *Inorg. Chem.* **1998**, *37*, 3644–3646.
- (13)  $[\text{VO}(\text{O}_2)(\text{NH}_2\text{-pyg}_2)]\text{K}$  is monoclinic,  $C2/c$ ,  $a = 25.840(8)$   $\text{\AA}$ ,  $b = 7.434(2)$   $\text{\AA}$ ,  $c = 16.713(5)$   $\text{\AA}$ ,  $\beta = 106.611(5)^\circ$ ,  $Z = 8$ .  $R = 3.3\%$ .  $[\text{VO}(\text{O}_2)(\text{BrNH}_2\text{-pyg}_2)]\text{K}$  is triclinic,  $P1$ ,  $a = 7.563(3)$   $\text{\AA}$ ,  $b = 14.446(5)$   $\text{\AA}$ ,  $c = 14.600(5)$   $\text{\AA}$ ,  $\alpha = 103.680(5)^\circ$ ,  $\beta = 102.750(5)^\circ$ ,  $\gamma = 91.286(5)^\circ$ ,  $Z = 4$ .  $R = 5.0\%$ .
- (14) Intermolecular H-bonding between crystallographically distinct molecules is shown. Bond lengths and angles of complex 2 (right, Figure 1ii) are essentially identical to those for complex 1 (left). See Supporting Information.
- (15) Butler, A. B.; Clague, M.; Meister, G. E. *Chem. Rev.* **1994**, *94*, 625.
- (16) The recently published structural characterization of  $[\text{VO}(\text{O}_2)\text{bpaH}]\text{ClO}_4 \cdot 2\text{H}_2\text{O}$  ( $\text{bpaH} = N,N$ -bis(2-pyridylmethyl)- $\beta$ -alanine) demonstrates an H-bond interaction between protonated carboxylate group and  $\text{V}(\text{V})$ -bound peroxo moiety, which is mediated by two water molecules that bridge the bpa donor and peroxo acceptor. However, as a result of equilibria between the structurally characterized complex and other  $\text{V}(\text{V})$  species in solution,  $[\text{VO}(\text{O}_2)\text{bpaH}]\text{ClO}_4 \cdot n\text{H}_2\text{O}$  is "not isolable in an analytically pure form". Casny, M.; Rehder, D. *Chem. Commun.* **2001**, *10*, 921.
- (17) Compounds are clean by  $^1\text{H}$  NMR in  $\text{CD}_3\text{CN}$  at and below room temperature, for ca. 24 h. In  $\text{D}_2\text{O}$  isomer ratios are approximately 85:15, with the major isomer consistent with  $\text{V}(\text{V})$  coordination as seen in the X-ray structures. Lability of  $\text{V}(\text{V})$  complexes has been addressed by Crans et al. See for example: Crans, D. C.; Chen, H.; Anderson, O. P.; Miller, M. M. *J. Am. Chem. Soc.* **1993**, *115*, 6769–6776.
- (18) Above room temperature both  $\text{NH}_2$  protons are equivalent by rotation, and appear upfield; thus the downfield-shifted  $\text{NH}_2$  proton is assigned as the H-bonding proton.



**Figure 2.**  $^1\text{H}$  VT NMR of  $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]^-$  in  $\text{CD}_3\text{CN}$ , showing pyridine region.  $\text{NH}_2$  protons diverge: from bottom to top at  $T =$  (a) 303, (b) 283, and (c) 233 K.

the intrinsic barrier to rotation of an amino group bound to an aromatic ring, ca. 6 kcal/mol,<sup>19</sup> is subtracted from the calculated  $\Delta G$ , then an H-bond strength of 6.4 kcal/mol can be estimated for  $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]\text{K}$ . This value is well

(19) Barriers restricting the internal rotation of  $\text{NH}_2$  in *N*-methylaniline, 2-aminopyridine, and 2-aminopyridine complexes (av) are ca. 6–7 kcal/mol,<sup>19a</sup> 4 kcal/mol, and 5.8 kcal/mol, respectively.<sup>19b</sup> (a) Anet, F. A. L.; Ji, X. *Tetrahedron Lett.* **1984**, 25, 1419. (b) Peris, E.; Lee, J. C., Jr.; Rambo, J. R.; Eisenstein, O.; Crabtree, R. H. *J. Am. Chem. Soc.* **1995**, 117, 3485.

within the range typical for H-bond strengths reported in the literature (2–10 kcal/mol).<sup>20</sup> Further investigations are in progress.

In summary, we have synthesized and structurally characterized complexes which demonstrate intramolecular H-bonding between amine and vanadium(V)-bound peroxide. The intramolecular H-bond strength in  $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]^-$  is estimated at 6 kcal/mol by  $^1\text{H}$  NMR studies and demonstrates that the H-bond interaction is significant. As such these complexes mimic the direct H-bond between  $\text{Lys}_{353}$  and the peroxo moiety in VCIPPO (*C. inaequalis*) and further support the notion that similar H-bonding occurs in VBrPO's.

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**Supporting Information Available:** Tables of NMR data and text giving experimental details and analytical data for  $\text{H}_2^{\text{NH}_2}\text{pyg}_2 \cdot 2\text{HCl}$ ,  $[\text{VO}(\text{O}_2)(^{\text{NH}_2}\text{pyg}_2)]\text{K}$ , and  $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]\text{K}$ , variable-temperature  $^1\text{H}$  NMR spectra for (18-crown-6)- $[\text{VO}(\text{O}_2)(^{\text{NH}_2}\text{pyg}_2)]\text{K}$  and - $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]\text{K}$ , and crystallographic data in CIF format for  $[\text{VO}(\text{O}_2)(^{\text{NH}_2}\text{pyg}_2)]\text{K}$  and  $[\text{VO}(\text{O}_2)(^{\text{BrNH}_2}\text{pyg}_2)]\text{K}$ . This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(20)  $T_{\text{coalescence}}$  is ca. 273 K for  $[\text{VO}(\text{O}_2)(^{\text{NH}_2}\text{pyg}_2)]\text{K}$ . A tentative value for the H-bond strength in  $[\text{VO}(\text{O}_2)(^{\text{NH}_2}\text{pyg}_2)]\text{K}$  is also about 6 kcal/mol. NMR studies will be performed in a solvent with lower freezing point.