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Modeling the Catalytic Site of Vanadium Bromoperoxidase: Synthesis and Structural Characterization of Intramolecularly H-bonded Vanadium(V) Oxoperoxo Complexes, $[VO(O_2)(^{NH_2}pyg_2)]K$ and $[VO(O_2)(^{BrNH_2}pyg_2)]K$

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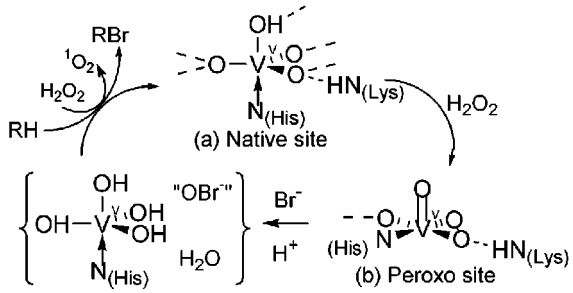
Vanadium haloperoxidases (VHPO) catalyze the peroxidative halogenation of organic substrates. Crystallographic studies suggest that hydrogen bonding from a lysine side chain to the vanadium(V)-bound peroxo group may facilitate oxidation of halides (Cl^-, Br^-, I^-). A ligand with pendant NH_2 functionality, *N*-(2-pyridylmethyl-6-amino) iminodiacetic acid ($H_2{}^{NH_2}pyg_2 \cdot 2HCl$) 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 $[VO(O_2)(^{NH_2}pyg_2)]K$ and $[VO(O_2)(^{BrNH_2}pyg_2)]K$ which demonstrate direct intramolecular H-bonding between an amine functionality and V(V)-bound peroxide is reported. The distances between NH_2 proton and bound peroxo moiety {(*d*(N(1)–H···O): 2.637(4) Å in $[VO(O_2)(^{NH_2}pyg_2)]K$, and 2.640(8) and 2.6919(8) Å in $[VO(O_2)(^{BrNH_2}pyg_2)]K$} are indicative of intramolecular H-bonding. The intramolecular H-bond strength in $[VO(O_2)(^{BrNH_2}pyg_2)]^-$ is estimated at 6 kcal/mol by ^1H 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).[1] The X-ray structures of vanadium chloroperoxidase from *Curvularia inaequalis*[2] and the vanadium bromoperoxidases from *Corallina officinalis*[3] and *Ascophyllum nodosum*[4] reveal that the vanadium active site resembles vanadate (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 conserved. Structural characterization of vanadium chloroperoxidase (VClPO) from *C. inaequalis* in the presence of H_2O_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).[5] In light of the importance of H-bonding in the regulation of metal ion reactivity,[6] a striking feature at the active site of VClPO is the apparent 2.76 Å H-bond between Lys_{353} 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_{398}, Lys_{349}, respectively) are also in positions to H-bond to a V(V)−peroxo moiety in VBrPO's.

Scheme 1. Proposed Catalytic Cycle for VHPO



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

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(1) (a) Butler, A.; Carter, J.; Simpson, M. In *Handbook on Metalloproteins*; Bertini, I., Sigel, A., Sigel, H., Eds.; Marcel Dekker Inc.: New York, Basel, 2001; pp 153−179. (b) Butler, A. *Coord. Chem. Rev.* **1999**, *187*, 17−35.

(2) Messerschmidt, A.; Wever, R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 392−396.

(3) Isupov, M. N.; Dalby, A. R.; Brindley, A. A.; Yoshikazu, I.; Tanabe, T.; Murshudov, G. N.; Littlechild, J. A. *J. Mol. Biol.* **2000,** *299*, 1035.

(4) Weyand, M.; Hecht, H.-J.; Kiess, M.; Liaud, M.-F.; Vilter, H.; Schomburg, D. *J. Mol. Biol*. **1999**, *293*, 595−611.

(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.

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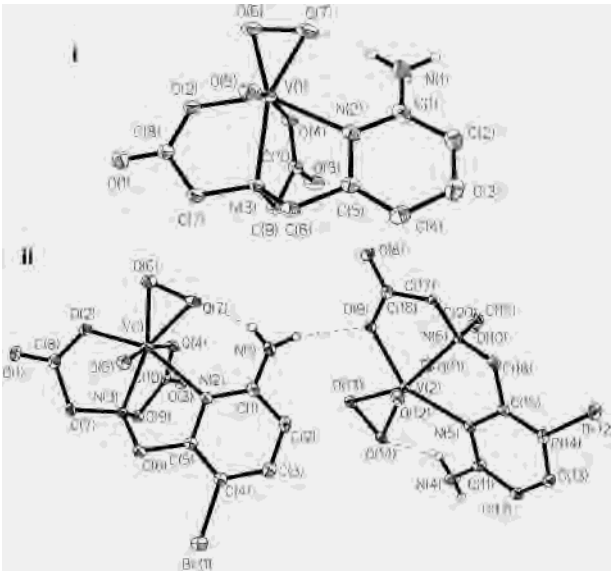
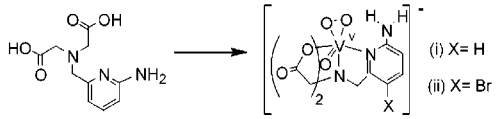


Figure 1. ORTEP views of $[VO(O_2)(^{NH_2}pyg_2)]^-$ (i) and $[VO(O_2)(^{BrNH_2}pyg_2)]^-$ (ii). Only NH_2 protons are shown for clarity. Selected bond lengths (Å) and angles (deg) for i: V−O(5) 1.608(2), V−O(6) 1.859(2), V−O(7) 1.880(2), O(6)−O(7) 1.419(2), N(1)−H···O(7) 2.637(4); O(5)−V(1)−N(2) 93.54(7), O(5)−V(1)−O(4) 166.30(7), O(5)−V(1)−O(7) 102.13-(7), O(5)−V(1)−O(6) 104.16, O(6)−V(1)−O(7) 44.61(8), N(1)−H−O(7) 144(3). For complex 1 of ii:[14] V(1)−O(5) 1.605(4), V(1)−O(6) 1.868(4), V(1)−O(7) 1.883(4), O(6)−O(7) 1.429(6), N(1)−H···O(7) 2.640(8), N(1)−H···O(9) 3.16; O(5)−V(1)−N(2) 91.5(2), O(5)−V(1)−O(4) 165.1(2), O(5)−V(1)−O(7) 103.1(2), O(5)−V(1)−O(6) 104.9(2), O(6)−V(1)−O(7) 44.8(2), N(1)−H···O(7) 125(4), N(1)−H···O(9) 156.

Scheme 2[a]



[a] (i) KVO_3, H_2O_2; (ii) KVO_3, H_2O_2, KBr.

protonation of the V(V)-bound peroxo group to generate a side-on bound hydroperoxide complex.[5] It has been proposed that an increase in positive charge on O_{peroxo}, by protonation, makes attack by halide more favorable.[7,8] 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, ($H_2{}^{NH_2}pyg_2$), with pendant NH_2 functionality.[9] We report the first structural characterization of VBrPO model complexes which demonstrate significant intramolecular H-bonding between a pendant amine functionality and V(V)-bound peroxide (Figure 1).[10]

$[VO(O_2)(^{NH_2}pyg_2)]K$ is obtained by adding $H_2{}^{NH_2}pyg_2{\cdot}2HCl$ to an aqueous slurry of KVO_3, followed by addition of H_2O_2 at pH 4 (Scheme 2i). In the presence of Br^- a bromoperoxidase type reaction occurs, giving $[VO(O_2)(^{BrNH_2}pyg_2)]K$, in which the pyridine ring is brominated para to the amine moiety (Scheme 2ii).[11,12] The molecular structures of $[VO(O_2)(^{NH_2}pyg_2)]K$[13] and $[VO(O_2)(^{BrNH_2}pyg_2)]K$ resemble those of other V(V)−peroxo complexes which are coordinated by tripodal, tetradentate amine ligands.[7] Both complexes have pentagonal bipyramidal structures with axial carboxylate O(4) and terminal oxo ligands. Peroxide binds symmetrically in the equatorial plane in a side-on fashion, with bond lengths typical for V(V)−oxo−monoperoxo complexes (d(V−O_{peroxo}) *ca*. 1.87 Å).[15] The distances between the amine nitrogen and bound peroxo moiety are indicative of intramolecular H-bonding: d(N(1)−H···O(7)) = 2.637(4) Å in $[VO(O_2)(^{NH_2}pyg_2)]K$; and d(N(1)−H···O(7)) = 2.640(8), d(N(1)−H···O(14)) = 2.62 Å in $[VO(O_2)(^{BrNH_2}pyg_2)]K$. It is noteworthy that the $LysN_{353}$−O(peroxo) bond length of 2.67 Å (*C. inaequalis*) is nearly the same.

To our knowledge $[VO(O_2)(^{NH_2}pyg_2)]K$ and $[VO(O_2)(^{BrNH_2}pyg_2)]K$ are the first models of VHPO's in which a direct intramolecular H-bond bridges an amine proton and a vanadium−peroxo moiety.[16] Variable-temperature ^1H NMR was used to investigate the integrity of the H-bonding (Figure 2).[17] At room temperature the 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 O(7).[18] In the case of $[VO(O_2)(^{BrNH_2}pyg_2)]K$ the NH_2 protons (6.5 and 8.0 ppm at 233 K) coalesce at *ca*. 283 K (500 MHz), corresponding to a ΔG^* value of 12.4 kcal/mol for rotation about the C−N bond. If

(7) Colpas, G. J.; Hamstra, B. J.; Kampf, J. W.; Pecoraro, V. L. *J. Am. Chem. Soc.* **1996**, *118*, 3469−3478.
(8) Butler, A.; Baldwin, A. H. *Struct. Bonding* **1997**, *89*, 109.
(9) Synthesis of $H_2{}^{NH_2}pyg_2{\cdot}2HCl$ 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 $[VO(O_2)(^{BrNH_2}pyg_2)]K$ were found and exhibit intermolecular H-bonding, as shown in Figure 1ii.
(11) Bromination of the ligand may be effected by $VO(O_2)(^{NH_2}pyg_2)]K$, or it could also occur via oxidation of Br^- by H_2O_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) $[VO(O_2)(^{NH_2}pyg_2)]K$ is monoclinic, *C*2/*c*, a = 25.840(8) Å, b = 7.434-(2) Å, c = 16.713(5) Å, β = 106.611(5)°, Z = 8. R = 3.3%. $[VO(O_2)(^{BrNH_2}pyg_2)]K$ is triclinic, *P*1, a = 7.563(3) Å, b = 14.446(5) Å, c = 14.600(5) Å, α = 103.680(5)°, β = 102.750(5)°, γ = 91.286-(5)°, 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 $[VO(O_2)bpaH]ClO_4{\cdot}2H_2O$ (bpaH = *N*,*N*-bis(2-pyridylmethyl)-β-alanine) demonstrates an H-bond interaction between protonated carboxylate group and V(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 V(V) species in solution, $[VO(O_2)bpaH]ClO_4{\cdot}nH_2O$ is "not isolable in an analytically pure form". Casny, M.; Rehder, D. *Chem. Commun.* **2001**, *10*, 921.
(17) Compounds are clean by ^1H NMR in CD_3CN at and below room temperature, for ca. 24 h. In D_2O isomer ratios are approximately 85:15, with the major isomer consistent with V(V) coordination as seen in the X-ray structures. Lability of V(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 NH_2 protons are equivalent by rotation, and appear upfield; thus the *downfield*-shifted NH_2 proton is assigned as the H-bonding proton.

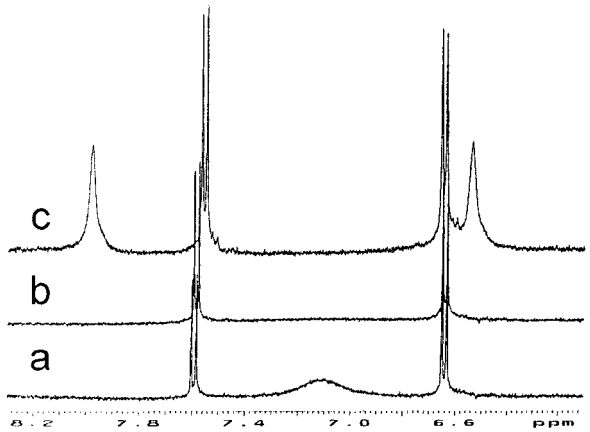


Figure 2. ^{1}H VT NMR of $[VO(O_2)(^{BrNH_2}pyg_2)]^-$ in CD_3CN, showing pyridine region. NH_2 protons diverge: from bottom to top at T = (a) 303, (b) 283, and (c) 233 K.

the intrinsic barrier to rotation of an amino group bound to an aromatic ring, ca. 6 kcal/mol,[19] is subtracted from the calculated ΔG, then an H-bond strength of 6.4 kcal/mol can be estimated for $[VO(O_2)(^{BrNH_2}pyg_2)]K$. This value is well within the range typical for H-bond strengths reported in the literature (2−10 kcal/mol).[20] 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 $[VO(O_2)(^{BrNH_2}pyg_2)]^-$ is estimated at 6 kcal/mol by ^{1}H NMR studies and demonstrates that the H-bond interaction is significant. As such these complexes mimic the direct H-bond between Lys_{353} and the peroxo moiety in VClPO (*C. inaequalis*) and further support the notion that similar H-bonding occurs in VBrPO's.

Acknowledgment. We are grateful for support from the National Science Foundation, Grant CHE9529374.

Supporting Information Available: Tables of NMR data and text giving experimental details and analytical data for $H_2{}^{NH_2}pyg_2 \cdot 2HCl$, $[VO(O_2)(^{NH_2}pyg_2)]K$, and $[VO(O_2)(^{BrNH_2}pyg_2)]K$, variable-temperature ^{1}H NMR spectra for (18-crown-6)-$[VO(O_2)(^{NH_2}pyg_2)]K$ and -$[VO(O_2)(^{BrNH_2}pyg_2)]K$, and crystallographic data in CIF format for $[VO(O_2)(^{NH_2}pyg_2)]K$ and $[VO(O_2)(^{BrNH_2}pyg_2)]K$. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) Barriers restricting the internal rotation of NH_2 in *N*-methylaniline, 2-aminopyridine, and 2-aminopyridine complexes (av) are ca. 6−7 kcal/mol,[19a] 4 kcal/mol, and 5.8 kcal/mol, respectively.[19b] (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.

(20) $T_{coalescence}$ is ca. 273 K for $[VO(O_2)(^{NH_2}pyg_2)]K$. A tentative value for the H-bond strength in $[VO(O_2)(^{NH_2}pyg_2)]K$ is also about 6 kcal/mol. NMR studies will be performed in a solvent with lower freezing point.