Reactivity of Dioxovanadium(V) Complexes with Hydrogen Peroxide: Implications for Vanadium Haloperoxidase

Brent J. Hamstra, Gerard J. Colpas, and Vincent L. Pecoraro*

Department of Chemistry, The University of Michigan, Ann Arbor, Michigan 48109-1055

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The dioxovanadium(V) complexes VO_2(bpg) (**1**), $[VO_2(pmida)]^-$ (**2**), and $[VO_2(ada)]^-$ (**3**) have been synthesized and characterized as models for the vanadium haloperoxidases. These compounds react with hydrogen peroxide in acetonitrile to form the corresponding peroxovanadium(V) complexes that have been previously studied by stopped-flow spectrophotometry. ^{1}H and ^{51}V NMR spectra of the VO_2^+ complexes in aqueous solution provide a clear picture of the solution structure of each complex. The results of these kinetic studies suggest an associative mechanism in which peroxide binds to a protonated form of the vanadium complex, followed by loss of a bound hydroxide or water molecule in the rate-determining step of the reaction and rapid rearrangement to the final product. The addition of acid to the reaction mixture results in rapid increases in the rate of peroxide binding by vanadium as a result of increased protonation of the complex. As in previous studies of similar reactions in aqueous solution, the reaction is first order in $[H^+]$ for substoichiometric amounts of acid, but when acid is present in excess, the dependence on $[H^+]$ becomes more complex, implicating the presence of hydroxide- and water-ligated intermediates. Under conditions in which no acid is added to the reaction mixture, the rate constants for formation of the peroxovanadium complex from the vanadium−peroxide adduct are 0.12 ± 0.04 s^{-1} for **1**, 0.33 ± 0.03 s^{-1} for **2**, and 0.29 ± 0.06 s^{-1} for **3**. The implications of this study with respect to catalysis by the vanadium-dependent haloperoxidase enzymes are discussed.

Introduction

Interactions of dioxygen and its reduced forms with transition metal ions represent an important class of reactions in chemical catalysis and bioinorganic chemistry. While iron (heme and non-heme), copper, and manganese ions are most commonly thought of when such reactions are considered, it has become clear that vanadium is also among the group of transition metals whose reactions with a reduced form of dioxygen are noteworthy in these fields. Peroxovanadium complexes have been shown to epoxidize alkenes,[1] hydroxylate a wide variety of hydrocarbons,[2] oxidize alcohols to aldehydes and ketones,[3] convert sulfides to sulfoxides and sulfones,[4] and perform other oxidations of organic and inorganic compounds.[5] They have also been shown to be potent insulin mimics, stimulating many of the effects associated with insulin activity but acting by a mechanism which, while unknown, appears to be quite different from that of insulin.[6,7] Such complexes may find use as new drugs for the treatment of insulin-dependent diabetics.

Vanadium haloperoxidases (VHPOs) have been isolated from several marine algae, fungi, and a lichen.[8−11] These enzymes catalyze the two-electron oxidation of chloride, bromide, and/or iodide by hydrogen peroxide and the halogenation of organic substrates and are likely to be involved in the biosynthesis of several compounds with potential medicinal uses.[11] Kinetic studies of the VHPOs are consistent with a mechanism in which the vanadium(V) ion first binds peroxide, and this peroxovanadium(V) complex then reacts with halide to generate an oxidized halogen species, which then may halogenate an organic substrate or, in the absence of substrate under basic conditions, react with additional hydrogen peroxide to form singlet oxygen.[12−14] Neither kinetic nor spectroscopic evidence suggests that the vanadium(V) ion is reduced during the catalytic cycle.

The active-site structures of the VHPOs have been probed by several spectroscopic and diffraction techniques. XAS and EXAFS measurements are consistent with a vanadium(V) ion which is ligated exclusively by oxygen and/or nitrogen donors, at least one of which is an oxo ligand.[15−17] EPR and ESEEM studies strongly suggest the presence of a nitrogen donor or donors (likely histidine imidazoles) to vanadium in the reduced,

* To whom correspondence should be addressed.

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inactive form of the enzyme.[18,19] Recently the crystal structures of native and peroxide-bound forms of vanadium chloroperoxidase were reported.[20,21] In the native form, the vanadium ion is bound to the protein solely by a histidine imidazole, and the rest of the donors to vanadium are either oxo or hydroxo ligands, which participate in numerous hydrogen-bonding interactions with nearby residues. The structure also reveals the presence of a histidine residue (His 404) near the active site which appears poised to act as an acid−base catalyst. In the peroxide-bound form, the histidine residue is no longer positioned as an acid−base catalyst, but lysine 353 is hydrogen-bonded to the bound peroxo ligand.[21] The peroxide-bound form also indicates that a vacant coordination site on vanadium is present, and it has been proposed that this serves as a binding site for chloride prior to its oxidation.

The structure of the native form is also strikingly similar to crystal structures obtained for vanadate-inhibited forms of rat acid phosphatase and a low molecular weight protein phosphotyrosine phosphatase.[22,23] In addition, Wever et al. recently showed that a vanadium-free form of a vanadium-dependent chloroperoxidase is competent to catalyze phosphate hydrolysis.[24]

Several functional models of VHPOs have been reported. The simplest of these is vanadate in acidic aqueous solution, which has been reported to oxidize bromide and iodide.[25,26] However, this system fails to reproduce the chemistry of the VHPOs in two key ways: (1) Appreciable halide oxidation in this system only occurs at pH values ≤ 2, as opposed to the enzymes which operate at or near neutral pH, and (2) the active catalyst in solution is a triperoxodivanadate species, rather than the mononuclear vanadium site observed in the enzymes. Butler and co-workers have demonstrated the ability of two vanadium(V)−Schiff base complexes to catalytically oxidize bromide in DMF under conditions in which only 1 equiv of acid is required per turnover.[27]

We recently reported the ability of several peroxovanadium complexes to rapidly and catalytically oxidize bromide and iodide in acetonitrile solution.[28] Kinetic and mechanistic studies of these complexes led us to propose the catalytic cycle for these complexes and the VHPOs shown in Figure 1.[29] In this mechanism, halide oxidation is proposed to occur via nucleophilic attack on bound peroxide without binding of halide directly to vanadium. While our experiments addressed the protonation, halide oxidation, and substrate halogenation steps of this cycle, the conversion of the dioxovanadium complex to the peroxovanadium complex remained unexplored under our experimental conditions. To study the last remaining step in this proposed catalytic cycle, we have synthesized and characterized a series of dioxovanadium complexes with some of the same ligands found in the above-mentioned peroxovanadium complexes and performed kinetic studies of their reactions with hydrogen peroxide to form the previously characterized peroxovanadium complexes.

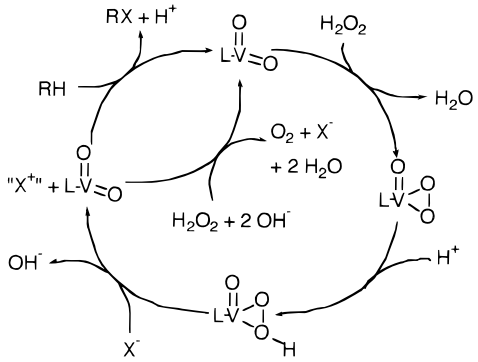

Figure 1. Proposed catalytic cycle for the vanadium haloperoxidases.

The reactions of similar complexes with hydrogen peroxide in acidic aqueous solution were previously studied by Wieghardt and by Tanaka et al.[30,31] Knowing that a change in solvent from water to acetonitrile significantly changed the chemistry of the peroxovanadium complexes, we wished to establish whether such a change in solvent would also have a significant effect on the reactivity of vanadium(V) with peroxide. The studies reported herein indicate that the kinetics of the conversion of dioxovanadium complexes to peroxovanadium complexes in acetonitrile, while quite similar to the kinetics observed in water, exhibit important differences which may shed new light on the ability of VHPOs to bind peroxide. Recognition of this difference may be relevant to the roles of peroxovanadium complexes as oxidation catalysts and insulin mimics.

Experimental Section

The following abbreviations are used throughout the text: Hbpg = *N*,*N*-bis(2-pyridylmethyl)glycine; H_2pmida = *N*-(2-pyridylmethyl)-iminodiacetic acid; H_2ada = *N*-(2-amidomethyl)iminodiacetic acid; H_3-nta = nitrilotriacetic acid.

Ethyl bromoacetate, 2-(aminomethyl)pyridine, 2-pyridinecarboxaldehyde, 2-picolyl chloride, iminodiacetic acid, H_2ada, sodium borohydride, sodium hydroxide, potassium hydroxide, vanadium(V) oxide, vanadyl sulfate, vanadyl bis(acetylacetonate), barium hydroxide, perchloric acid, potassium nitrite, and 18-crown-6 were purchased from Aldrich Chemical Co. Acetonitrile was purchased from Mallinckordt Chemical Co. Hydrogen peroxide was purchased from Fisher. Methanol was purchased from EM Science. Ethanol was purchased from McCormick Distilling Co.

Preparation of Compounds. The syntheses of Hbpg·2HBr, [VO(H$_2$O)pmida]·2H$_2$O, and [VO(H$_2$O)ada]·2H$_2$O were reported previously.[32−34] Sodium and potassium vanadate were generated in situ by the reaction of V_2O_5 with 2 equiv of the appropriate hydroxide in aqueous solution.

VO$_2$bpg·2H$_2$O (1)·2H$_2$O. Hbpg·2HBr (0.46 g, 1.1 mmol) was added to a solution of vanadate (1.1 mmol) in 10 mL of H_2O with stirring. The solution immediately became a deep reddish-orange color and was adjusted to pH 9. Upon cessation of stirring and evaporation of solvent, red crystals formed within 2 days. The crystals were filtered

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from the solution and air-dried. Additional crystals formed upon further evaporation of solvent. Yield = 0.15 g (38%). Anal. Calcd (found) for $C_{14}H_{14}N_3O_4V\cdot 2H_2O$: C, 44.81 (44.53); H, 4.83 (4.96); N, 11.20 (11.34). Infrared spectrum (ν, cm^{-1}): 1626, C=O; 905, 889, V=O. ^1H NMR (D_2O): δ 3.75 (2H, s), 4.56 (2H, d, J = 15 Hz), 5.23 (2H, d, J = 16 Hz), 7.68 (2H, m), 8.16 (1H, m), 8.55 (1H, d, J = 4 Hz). ^{51}V NMR (CD_3CN): −494 ppm.

K[VO_2pmida]·CH_3OH (2)·CH_3OH. [$VO(H_2O)$pmida]·$2H_2O$ (1.72 g, 5 mmol) was ground to powder and suspended in 50 mL of 1:2 H_2O/MeOH. The suspension was stirred vigorously over low heat, and KNO_2 (0.85 g, 10 mmol) was added slowly in portions with continued stirring. As the reaction progressed, the solids dissolved with evolution of gas and the color of the solution changed from blue to dark blue to green to yellow over the course of 3−4 h. Upon cooling, a yellow powder precipitated from solution. The mixture was filtered, and the precipitate was immediately transferred to a desiccator for drying. Yield = 0.78 g (41%). Anal. Calcd (found) for $C_{10}H_{10}N_2O_6KV\cdot CH_3OH$: C, 35.11 (35.21); H, 3.75 (3.40); N, 7.44 (7.84). Infrared spectrum (ν, cm^{-1}): 1669, 1620, C=O; 918, 899, V=O. ^1H NMR (D_2O): δ 3.63 (1H, d, J = 18 Hz), 3.75 (1H, d, J = 19 Hz), 3.87 (1H, d, J = 16 Hz), 4.48 (1H, d, J = 15 Hz), 4.53 (1H, d, J = 16 Hz), 4.89 (1H, d, J = 14 Hz), 7.61 (2H, m), 8.09 (1H, m), 8.53 (1H, m). ^{51}V NMR (D_2O): −510 ppm.

K[VO_2ada]·H_2O (3)·H_2O. [$VO(H_2O)$ada]·$2H_2O$ (1.55 g, 5 mmol) was ground to powder and suspended in 50 mL of 1:2 H_2O/MeOH. The suspension was stirred vigorously over low heat, and KNO_2 (0.85 g, 10 mmol) was added slowly in portions with continued stirring. As the reaction progressed, the solids dissolved with evolution of gas and the color of the solution changed from blue to dark blue to green to yellow over the course of 3−4 h. Upon cooling, a yellow microcrystalline product precipitated from solution. The mixture was filtered, and the precipitate was allowed to air-dry. Yield = 1.05 g (63%). Anal. Calcd (found) for $C_6H_8N_2O_8KV\cdot H_2O$: C, 21.96 (22.05); H, 3.07 (2.90); N, 8.54 (8.51). Infrared spectrum (ν, cm^{-1}): 1682, 1631, C=O; 911, 877, V=O. ^1H NMR (D_2O): δ 3.84 (2H, d, J = 17 Hz), 4.05 (2H, s), 4.24 (2H, d, J = 15 Hz). ^{51}V NMR (H_2O): −506 ppm.

Kinetic Studies. All reactions were carried out in acetonitrile. Potassium salts of vanadium compounds were dissolved in acetonitrile by the addition of 2 equiv of 18-crown-6 for each potassium ion and were made fresh daily as needed. Stock hydrogen peroxide solutions were made by adding a known quantity of 50% H_2O_2 to acetonitrile, and the solutions were standardized with permanganate and stored in a dark refrigerator. More dilute solutions for use in the experiments were generated from the stock solution as needed. Acidic peroxide solutions (if needed) were made by adding known quantities of concentrated $HClO_4$ to freshly generated peroxide solutions as needed. Reactions were initiated by the mixture of a solution containing the vanadium complex with a solution containing hydrogen peroxide (or peroxide and acid when experiments using excess acid were performed).

All reactions were performed on an OLIS RSM-SF rapid-scan stopped-flow spectrophotometer at a temperature of 25.0 ± 0.1 °C, and the resulting data were fit using the software package provided with the OLIS RSM-SF. Initial rate data (≤20% of reaction) were fit to a line using the general equation $y = mx + b$, and reaction curves were obtained under pseudo-first-order conditions for the general equation $y = a\exp(bx) + c$. Rates obtained by these methods were compared to each other and to the results of global fitting routines also found in the software package provided with the OLIS RSM-SF, and all were consistent within experimental error. Kinetic parameters and equilibrium constants were obtained from plots of observed rates vs concentrations and fit using the curve-fitting routines in the program Kaleidagraph.

Physical and Spectroscopic Studies. Infrared spectra were recorded as KBr pellets on a Nicolet 60 SX Fourier transform spectrophotometer. UV/visible spectra were recorded on a Perkin-Elmer Lambda 9 spectrophotometer. NMR spectra were recorded using a Bruker 200 MHz instrument. ^1H NMR were referenced to internal DSS (0.0 ppm). ^{13}C NMR were referenced to DSS (0.0 ppm) using dioxane as an internal reference. ^{51}V NMR were referenced to external neat $VOCl_3$ (0.0 ppm). Elemental analyses were performed by the University of Michigan Microanalysis Laboratory.

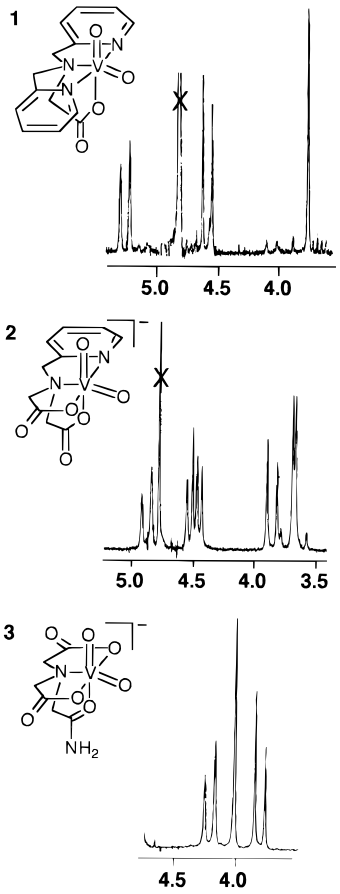

Figure 2. ^1H NMR spectra and proposed solution structures for **1**−**3** in aqueous solution. Peaks marked with an × are attributed to H_2O.

Results

Synthesis. Syntheses of complexes **1**−**3** were straightforward. While **1** was easily prepared from an aqueous vanadate solution, due to its relatively low solubility, this method was not as successful for the other complexes. This may be due to the ability of vanadate to form a variety of complexes with amino carboxylate ligands of this type depending on concentration and pH as previously demonstrated by Crans and coworkers.[35] Consequently, we turned to the oxidation of the corresponding vanadium(IV) complexes by nitrite as had been previously demonstrated by Saito et al. for the synthesis of similar complexes.[36,37]

Solution Structures of 1−3. NMR spectroscopy was used to probe the structures of **1**−**3** in solution. For each of the complexes, ^{51}V NMR spectra contained only one peak and were inconsistent with the presence of free vanadate in solution. The chemical shift value of −506 ppm reported for **3** is identical to that reported previously for this complex by Crans and Shin.[35]

^1H NMR spectra of the complexes in aqueous solution (Figure 2) gave clear evidence that in each case the ligand remained bound to vanadium in solution. This was most evident in the regions of the spectrum in which resonances due to methylene protons were observed. As indicated in Figure 2 and in the Experimental Section, doublets with J values of 14−19 Hz were commonly observed. Such values are consistent with couplings between chemically inequivalent geminal protons. For each complex, only one orientation of the ligand with respect to the

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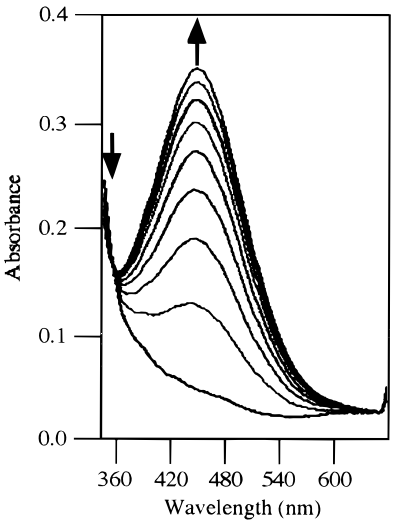

Figure 3. Series of spectra illustrating growth of the peroxo-to-vanadium charge-transfer band as hydrogen peroxide reacts with **2**. Conditions used: 0.5 mM $[VO_2(pmida)]^-$, 90 mM H_2O_2. Spectra shown were collected at equal intervals between 1.5 and 40 s from the initiation of the reaction.

VO_2^+ unit is consistent with the spectral data. For **1** and **3**, this is an arrangement in which the pyridine donors (for **1**) or the carboxylate donors (for **3**) are coordinated trans to each other, leaving the other donors in the positions trans to the oxo ligands. For **2**, in which each methylene proton is shown to be inequivalent, an asymmetric orientation which leaves the tertiary amine and one of the two carboxylates trans to the oxo moieties is dictated. These structural assignments are also consistent with the ^{13}C NMR spectra, since for each complex the resonances observed for the methylene carbons are shifted downfield from those observed for the free ligand, and there are an appropriate number of resonances consistent with the geometries inferred from the ^1H NMR studies (2 for **1** and **3**; 3 for **2**). These spectra may also indicate the presence of an equilbrium in which the more weakly bound donor trans to the oxo ligand dissociates from vanadium, while the rest of the donors remain tightly bound under the experimental conditions.

Kinetic Studies. The reaction of **2** with H_2O_2 is illustrated in Figure 3. These spectra are representative of the changes in the UV−visible spectra observed for each of the dioxovanadium complexes upon reaction with peroxide in acetonitrile. Isosbestic behavior was observed for each complex as the absorbance due to the peroxo-to-vanadium charge-transfer band of the product complex increased, providing a convenient spectroscopic handle for the measurement of the kinetics of formation of the peroxovanadium complexes. The reactions of **1** and **3** with peroxide yielded spectra which were identical to previously measured spectra of structurally characterized peroxovanadium complexes with these ligands in acetonitrile, providing proof of the identity of the products of the reactions.[29]

For each complex, the initial rate of formation of the peroxovanadium complex was found to be directly proportional to the concentration of the dioxovanadium complex in solution. This is best illustrated for **2**, as shown in Figure 4. For each complex, the dependence remained linear for approximately an order of magnitude range in the vanadium concentration. The data were fit to straight lines, and the slopes of these lines yielded pseudo-first-order rate constants, which upon correction for the peroxide concentration, yielded second-order rate constants ranging from 0.094 ± 0.042 M^{-1} s^{-1} for **1** to 0.44 ± 0.22 M^{-1} s^{-1} for **3** and 0.47 ± 0.24 M^{-1} s^{-1} for **2**.

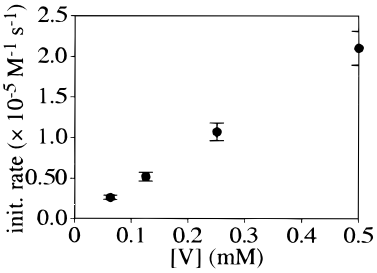

Figure 4. Graph illustrating dependence of the initial rate of peroxovanadium complex formation on the concentration of **2** in solution. Condition used: 90 mM H_2O_2.

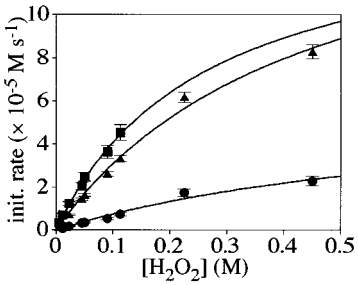

Figure 5. Graph illustrating dependence of the initial rate of peroxovanadium complex formation on the concentration of H_2O_2 in solution. Condition used: 0.5 mM V. Values of rate and equilibrium constants derived from the curve fits shown are given in Table 1. Key to figure: **1**, circles; **2**, triangles; **3**, squares. The range of concentrations used for **3** was limited by diminished stability of the complex in the presence of high concentrations of peroxide.

Table 1. Equilibrium and Rate Constants for the Reaction of H_2O_2 with **1**−**3** in the Absence of Added Acid

complex	K (M^{-1})	k_2 (s^{-1})
1	1.4 ± 0.6	0.12 ± 0.04
2	2.3 ± 0.4	0.33 ± 0.03
3	3.9 ± 1.0	0.29 ± 0.06

The dependence of the initial rate of peroxovanadium complex formation on the concentration of peroxide in solution exhibited saturation behavior for all of the complexes studied, as illustrated in Figure 5. The data were therefore fit to a model in which a preequilibrium step precedes the formation of the peroxovanadium complex, as illustrated in eqs 1 and 2. In this

$$VO_2L + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} VO_2L{\cdot}H_2O_2 \quad (1)$$

$$VO_2L{\cdot}H_2O_2 \overset{k_2}{\rightleftharpoons} VO(O_2)L \quad (2)$$

model, no assumptions regarding the composition and structure of the intermediate complex are made other than that peroxide is in some way associated with the complex. Given this model, eq 3 then describes the rate of formation of the final product in

$$d[VO(O_2)L]/dt = (k_2K[VO_2L]_0[H_2O_2]_0)/(1 + K[H_2O_2]_0) \quad (3)$$

terms of the equilibrium constant for formation of the intermediate ($K = k_1/k_{-1}$) and rate constant (k_2) for the conversion of this intermediate to the final product under the conditions of these experiments, where $k_2 << k_{-1}$ and $[H_2O_2]_0 >> [VO_2L]_0$.

The rate and equilibrium constants determined by least-squares regression using eq 3 to fit both rate and equilibrium constants are given in Table 1. The first-order rate constants determined from these data compare favorably in magnitude

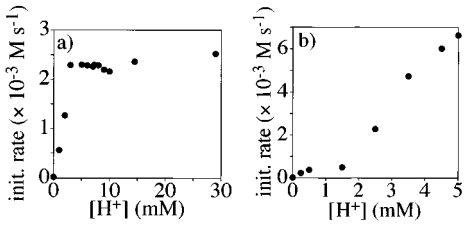

Figure 6. Graphs illustrating dependence of the initial rate of peroxovanadium complex formation on $[H^+]$ (added as $HClO_4$). (a) Conditions used: 2.5 mM $[VO_2(bpg)]$, 90 mM H_2O_2. (b) Conditions used: 0.5 mM $[VO_2(ada)]^-$, 90 mM H_2O_2.

with those rate constants extracted from the vanadium-dependence experiments.

The addition of perchloric acid to the reaction mixture resulted in a marked increase in the initial rate of the reaction. In these experiments, a solution of the vanadium complex was reacted with a mixture of acid and peroxide because the alternative order of addition of reactants resulted in undesirable side reactions. Addition of acid to an acetonitrile solution of **1**, **2**, or **3** caused the formation of a yellow precipitate, and the characterization of the product(s) of this reaction remains a subject of continuing study. The remaining acidic solutions showed signs of further decomposition of the remaining complex in solution (possibly due to reduction of vanadium) over the course of hours. No precipitation or apparent decomposition was observed when acidic peroxide solutions were added to solutions of the vanadium complexes.

Figure 6a illustrates the change in initial rate as a function of $[H^+]$ for **1**. While even substoichiometric acid concentrations were shown to produce an increase in the rate of reaction, virtually no increase in rate was observed when greater than 1 equiv of acid per vanadium complex was added. These results suggest that a single protonation of the dioxovanadium complex is sufficient to significantly accelerate the rate of peroxovanadium complex formation. For **2** and **3**, however, the acid dependence of the reaction was significantly more complex, as illustrated for **3** in Figure 6b. For these complexes, a significant increase in rate is observed for $[H^+] <$ [complex]. At slightly greater than stoichiometric $[H^+]$, little increase in rate is observed, and then another greater increase in rate is observed as $[H^+]$ increases further. The results are consistent with the contribution of two species in different protonation states to the reaction under these conditions, with one species predominant at lower concentrations of acid and the other predominant at higher concentrations. Experiments at higher $[H^+]$ than those presented here could not be reliably carried out due to the instability of the peroxovanadium product under such acidic conditions.

In addition, experiments designed to examine the peroxide dependence of the reaction under these acidic conditions (data not shown) revealed that the saturation behavior previously observed when no acid was added to the solution either was greatly diminished or disappeared entirely, and the peroxide dependence appeared to revert to simple first-order behavior. No attempt was made to fit data obtained in these experiments to the preequilibrium model described above.

Discussion

Our previous studies of the chemistry of peroxovanadium-(V) complexes in acetonitrile indicated that while the complexes' structures remained the same in the solid state and in both aqueous and acetonitrile solutions, the reactivity of these complexes was significantly affected upon changing from aqueous to acetonitrile solution. The results of this study of the solution structures and reactivity of similar dioxovanadium-(V) complexes show that these conclusions are valid for complexes of this type as well.

Although the complexes used in this study have not been crystallographically characterized, the NMR spectra of the complexes provide a clear picture of their structures in solution. For each of the compounds, ^1H NMR spectra are consistent with only one coordination mode for the amino carboxylate ligand and suggest that, under the conditions of the kinetic experiments, ligand exchange is relatively slow (with the possible exception of ligands coordinated trans to oxo ligands). Similar results for other complexes of this type have been reported by other investigators.[38] A comparison of the solution structures obtained in this work for **1** and **3** to the crystal and solution structures for the complexes $VO(O_2)bpg$ and $[VO(O_2)ada]^-$ indicates that the structures of the complexes are the same with respect to the arrangement of ligands and differ only by the substitution of a peroxo ligand for an oxo ligand in the latter pair of complexes.[29]

The proposed structural similarities between the dioxovanadium and peroxovanadium complexes are also supported by the isosbestic behavior observed upon the reactions of the dioxovanadium complexes with peroxide (Figure 3). This observation mitigates against the existence of any major changes in the coordination environment of vanadium during the course of the reaction unless these changes are rapid enough not to be observed on the stopped-flow time scale.

Previous studies of the formation of peroxovanadium complexes from dioxovanadium precursors in aqueous solution by Tanaka et al. revealed the presence of two separate reaction pathways, both of which are first-order in vanadium and peroxide.[31] The difference in the two proposed pathways is that one pathway is acid-independent, while the other pathway exhibited a first-order dependence on $[H^+]$ over the range of concentrations used in that study (pH 1.5−5). Wieghardt obtained similar results under these conditions for the complex $[VO_2(dipic)H_2O]^-$ (dipic: dianion of pyridine-2,6-dicarboxylic acid), although the kinetics were apparently complicated by trimerization of the complex and a more complex dependence on $[H^+]$ at extremely low pH values (pH $\leq$1) was observed.[30] Both Wieghardt and Tanaka et al. implicated an associative mechanism in their studies: Wieghardt on the basis of an observed difference in rates associated with a change in the identity of the nucleophile (H_2O_2 or HO_2^-) and Tanaka et al. because of the large negative values of $\Delta S^\ddagger$ they determined for the reaction (for $[VO_2(nta)]^{2-}$, $\Delta S^\ddagger = -96 \pm 9$ J K^{-1} mol^{-1} for the acid-independent pathway).[30,31] The results obtained in the present study largely corroborate these findings, although some important differences are observed.

In this study, as in those mentioned above, the dependence of the rate of peroxovanadium complex formation on the concentration of vanadium in solution is first-order. However, the simple first-order dependence of the reaction on peroxide is not observed in the absence of added acid, although the addition of excess acid to the solution does result in kinetics which show a first-order dependence on peroxide concentration as observed in the previous studies. It should be noted that the range of peroxide concentrations used in this study is larger than that employed in previous work.

(38) Crans, D. C.; Shin, P. K.; Armstrong, K. B. Application of NMR Spectroscopy to Studies of Aqueous Coordination Chemistry of Vanadium(V) Complexes. In *Mechanistic Bioinorganic Chemistry*; Thorp, H. H., Pecoraro, V. L., Eds.; American Chemical Society: Washington, DC, 1995; pp 303−328.

The major difference from our work is in the more complex rate dependence on $[H^+]$ when $[H^+] \geq [V]$. While at substoichiometric acid concentrations a first-order acid dependence is observed for all complexes as in the previous studies, the insensitivity of the reaction to the concentration of acid under more acidic conditions for **1** and the complex behavior for **2** and **3** are in stark contrast to previous results. This difference is most probably attributable to the change in solvent from water to acetonitrile. Ions and molecules which are negligibly basic in aqueous solution due to the leveling effect of water may exhibit considerable basicity in acetonitrile. As a consequence, we previously discovered that the reactivity of peroxovanadium complexes toward bromide oxidation was enormously enhanced upon a change in solvent from water to acetonitrile, due to the fact that in acetonitrile the vanadium complex did not have to compete with water molecules for protons.[29] A similar effect is probably operative here. Whereas in aqueous solution the vanadium complex may be much less basic than water, and consequently most protons in solution are bound as H_3O^+, in acetonitrile the competing base (water) is removed and/or the relative basicities of the complexes are increased and efficient protonation of the complex can occur. Since the protonation equilibrium lies nearly completely in favor of the initial protonation of the complex, excess acid does little, if anything, to generate the first protonated complex which must be involved in the rate-limiting step of the reaction and, therefore, no rate dependence on $[H^+]$ is observed at higher concentrations. For **2** and **3** evidence of a second more difficult protonation is observed. The realizations that the form of acid used in these experiments is H_3O^+, not just H^+, and that water can act as a very weak conjugate base under these conditions (pK_a for H_3O^+ in $CH_3CN \approx 2.2$) become important to consider.[39] The results are consistent with the formation of an intermediate whose acidity is comparable to the hydronium ion in acetonitrile solution.

Protonation of the complex is also consistent with the ability of the peroxide-binding reaction to occur in the absence of added acid. In this case, hydrogen peroxide acts as a weak acid and the protonation equilibrium is shifted toward proton transfer from peroxide to the complex by the rapid conversion of the protonated complex to the peroxovanadium product.

A mechanism for the reactivity of **1**−**3** with peroxide which is consistent with previous studies and accounts for all of the kinetic observations discussed above is given in Figure 7. In this mechanism, the dioxovanadium complex is protonated (by hydrogen peroxide if no excess acid is present), leaving an oxohydroxovanadium complex. Such complexes, although not directly observed in this study, were previously observed and characterized by several investigators.[40,41] Hydrogen peroxide (or, alternatively, a hydroperoxide ion) can then coordinate to vanadium in an associative mechanism, forming a seven-coordinate intermediate as implied by the observed saturation behavior. At higher acid concentrations, protonation of the hydroxo ligand may occur, yielding a bound water molecule. Coordinated hydroxide or water then dissociates in the rate-determining step. Tanaka and co-workers, in a previous study of the reaction of the VO_2^+ ion with ethylenediamine-*N*,*N*′-diacetic acid, showed that the rate-determining step of the reaction was water dissociation from the vanadium ion.[42] Following the dissociation step, rapid ring closure by the peroxo ligand results in the peroxovanadium product. Wieghardt suggested, on the basis of his studies of $[VO_2(dipic)H_2O]^-$, that the intramolecular rate constant for ring closure of the peroxo ligand should be approximately 10^7-10^9 s^{-1}, much higher than the lowest rate constant observed in this study.[30] Since the addition of acid to this system results in an acceleration of the rate of reaction by facilitating protonation of bound oxo and/or hydroxo ligands, the saturation behavior with respect to peroxide is minimized or disappears as the hydroxo- and aquo-ligated species are produced and react at sufficient rates to prevent appreciable accumulation of the seven-coordinate intermediate.

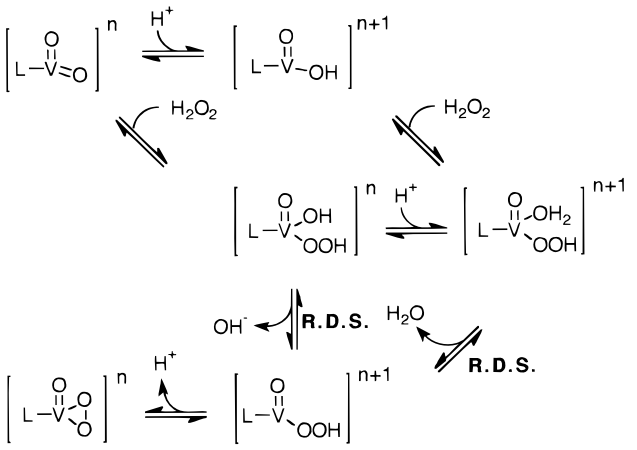

Figure 7. Proposed mechanism for peroxovanadium complex formation based on the results of this study.

This mechanism for peroxide binding is similar to that proposed for the vanadium chloroperoxidase by Messerschmidt et al., in that it recognizes the importance of the protonation of oxo ligands in the binding of peroxide. However, their mechanism differs from that proposed here (and from that proposed in the studies described above) in that they suggest that the binding of peroxide takes place via a dissociative mechanism, in which water dissociates from vanadium prior to the binding of hydroperoxide.[21] The structural and kinetic data they report for the chloroperoxidase are consistent with the associative model proposed on the basis of this work as well, and this associative mechanism has the advantage of being consistent with several modeling studies. A second difference in the mechanisms proposed by Messerschmidt and co-workers and that which we have previously proposed for the vanadium-dependent haloperoxidases[29] is that their mechanism invokes vanadium-bound halide species, whereas our mechanism does not include such species. Either of these alternatives is consistent with the peroxide-binding data presented here.

At this point, an explanation can be given for the difference in observed acid dependence for **1** when compared to that of **2** and **3**. Whereas **2** and **3** possess a negative charge, **1** is neutral. A single protonation of each of these complexes yields neutral complexes in the case of **2** and **3** and a positively charged complex for **1**. A second protonation of **1** would yield a complex containing a +2 charge. The addition of this second positive charge on the complex may well be strongly thermodynamically disfavored in acetonitrile as compared to the addition of the first charge. Consequently, the second protonation of **1** is not observed or is negligible under the current experimental conditions. In contrast, a second protonation of the less positively charged complexes **2** and **3** can occur.

In our study of the catalytic oxidation of halides by peroxovanadium complexes, we suggested that dioxovanadium com-

(39) Izutsu, K. *Acid*−*base dissociation constants in dipolar aprotic solvents*; Blackwell Scientific Publications: Oxford, U.K., 1990.
(40) Giacomelli, A.; Floriani, C.; Duarte, A. O. d. S.; Chiesi-Villa, A.; Guastini, C. *Inorg. Chem.* **1982**, *21*, 3310.
(41) Li, X.; Lah, M. S.; Pecoraro, V. L. *Inorg. Chem.* **1988**, *27*, 4657.
(42) Yamada, S.; Ukei, Y.; Tanaka, M. *Inorg. Chem.* **1976**, *15*, 964.

plexes are formed upon oxygen atom transfer from vanadium-bound hydroperoxide to halide in acetonitrile and that these complexes could then react with peroxide to regenerate peroxovanadium complexes (Figure 1). The current study indicates that the formation of peroxovanadium complexes from dioxovanadium complexes in acetonitrile is facile and that this hypothesis was reasonable. Each step in the proposed catalytic cycle has now been individually examined, and rate constants have been determined for the peroxovanadium complex formation and halide oxidation reactions. From the data presented here, it appears that in the case of the model compounds peroxovanadium complex formation may be rate-limiting. For **1**, the second-order rate constant for the conversion to $VO(O_2)$-bpg under conditions where ≥ 1 equiv of acid is present can be estimated from the data in Figure 6a to be roughly 11 ± 2 M^{-1} s^{-1}. The rate constant reported for bromide oxidation by $VO(O_2)$bpg is 21 ± 3 M^{-1} s^{-1}.[29] Given that bromide might act as a competing base to inhibit peroxide binding to vanadium as it appears to act in inhibiting protonation of bound peroxide, the formation of peroxovanadium complexes may be slowed under turnover conditions, where excess bromide is present. Kinetic studies of the reactivity of the complexes with peroxide and halides under catalytic conditions will be necessary to evaluate this potential role of bromide and other halides as inhibitors in addition to their previously studied role as substrates.

With respect to catalysis by the VHPOs, this study suggests that acid−base catalysis may be important in the catalytic cycle not only for the protonation and activation of bound peroxide toward oxidation, as suggested by previous studies, but also in facilitating the binding of peroxide to vanadium(V) by labilizing oxo and/or hydroxo ligands as well. While on the basis of crystallographic studies histidine 404 is the obvious choice for the essential acid−base catalyst for both the peroxide binding and peroxide activation steps of the reaction, the crystal structure of the peroxide-bound form indicates that lysine 353 is hydrogen-bonded to the peroxo ligand. Regardless of what specific residues may be involved in the catalytic cycle, whether peroxide binds via an associative or dissociative mechanism, or whether halide ions coordinate directly to vanadium, it is clear that protonation of oxo/hydroxo and peroxo ligands bound to vanadium is consistent with the emerging picture of the reactivity of these enzymes.

The current results also suggest that the relative basicity of oxo ligands may be increased in a relatively less polar environment. While in the present study this environmental change is brought about by the change of solvent from water to acetonitrile, in the enzyme this may be done by sequestering the vanadium ion in a relatively nonaqueous environment in the interior of the protein. The recent crystal structure of the vanadium chloroperoxidase reveals that vanadium is bound in just such a hydrophobic site.[20] This study also suggests that it may be necessary to maintain an active-site environment that is nearly charge-neutral, as a buildup of positive charge may result in inhibition of complex protonation.

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