The Chemistry and Biochemistry of Vanadium and the Biological Activities Exerted by Vanadium Compounds

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1. Introduction

Vanadium is a trace element, which may be beneficial and possibly essential in humans[1] but certainly essential for some living organisms.[2−11] Metal ions and thus vanadium ions can play a role in biology as counterions for protein, DNA, RNA, and in various biological organelles. The structural role is often manifested by the maintenance of various biological structures, whereas a functional role is to bring key reactivity to a reaction center for a protein. Vanadium ions have many structural roles reflected by its structural and electronic analogy to phosphorus.[9,12−20] In addition, the vanadium ion is an enzyme cofactor,[7,9,21−30] and is found in certain tunicates[4−11,16] and possibly mammals.[1] Reviews on how vanadium can act and function in the biosphere include investigations into the fundamental coordination and redox chemistry of the element,[16−18,29,31−35] as well as structural and functional aspects of biological systems and/or metabolites.[12,36] Modeling biological activities of various types have long been of interest to chemists, with this discipline focusing on the structural modeling until about a decade ago when the focus shifted to functional modeling. Clearly modeling that includes both aspects will be most informative, and the ultimate goals for model chemists. Although the latter in general may be of greater interest at the present time, the structural aspects of the various oxidation states are defining its effects in many biological systems. In this review, we have combined the two fundamentally different aspects of modeling because the coverage of only one of these areas in our opinion would not provide the reader the proper sense of the effects and activities exerted by vanadium compounds (V-compounds).

This review describes the voyage from the discovery of the first vanadium-containing enzymes in 1984, haloperoxidases,[7,9,21−26,37,38] to the current X-ray crystallographic studies; it is a fascinating story that provides inspiration to chemist in many fields. These studies map out the enzyme active site[24,39−43] and demonstrate the structural and functional link between apohaloperoxidases and certain phosphatases.[24,39−43] These discoveries now give the bioinorganic chemists clear directives. The detailed mechanistic studies revealing how the enzyme-catalyzed reaction takes place,[7,22,23,37,38] to the design of simple vanadium complexes (V-complexes) exhibiting similar activities as the enzyme show how much the chemists can do.[7,9,29,38,44−48] Inhibition of phosphatases by V-compounds is firmly established,[9,12−15,19,20] and often the versatility of the vanadium to bind as a four-coordinate ground state analogue and a five-coordinate transition state analogue is not generally recognized; thus, V-compounds can act as substrates although bioinorganic chemists are more familiar with the five-coordinate transition state analogy as exemplified in the potent inhibition of phosphatases, ribonuclease,[49,50] and other phosphorylases. Although

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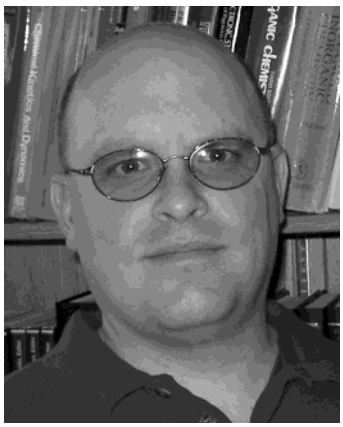


Jason J. Smee received his B.Sc. at Kansas State University in 1994. He then pursued a Ph.D. under the direction of Marcetta Y. Darensbourg at Texas A&M University in College Station, Texas. After graduating in 2000, he moved on to Colorado State University where he is a postdoctoral associate with Debbie C. Crans. His research focuses on the synthesis and characterization of novel vanadium complexes as potential therapeutic drugs in the treatment of diabetes. In his spare time he enjoys reading, playing softball, and long bike rides.

Ernestas Gaidamauskas, born in 1971 in Vilnius, Lithuania, received his Ph.D. in physical chemistry at the Vilnius University in 1999. From 2000 to 2003, he undertook postdoctoral studies at the Colorado State University under the supervision of Professor Debbie C. Crans. In 2003, Ernestas was appointed to his current position of lecturer and research associate at Vilnius University. His research interests include the metal speciation with various bioligands and redox chemistry of metal complexes.

the second V-containing enzyme, the V-nitrogenase,[7,27,28,51] is not as well understood as the vanadium-containing haloperoxidases, it is currently a target for both mechanistic[7,27−29] and modeling[52,53] studies with novel structural information constantly modifying the current understanding this system. Combined, these studies bode well for new classes of V-containing proteins, the vanabins,[2,5,10,11] which have been proposed to be transport proteins for vanadium, and promise exciting developments in the future.

The elucidation of the structure of the vanadium-containing natural product, amavadine,[54] remains an interesting structural investigation,[29,55−58] although questions now being addressed in this area have shifted to the investigations of this complex's catalytic properties. Perhaps these developments will provide additional clues to the function of this unique compound. The investigations into tunicate metabolism and biology,[2,3,11,59,60] the recognition of the important role of sulfate in the intact blood cells, and the firm documentation that V(III) does exist in some species as well as the discovery of a V-containing transport protein present key developments to understanding why and how these organisms contain vanadium. Finally, the boom of investigations into the insulin-like action of V-complexes documents the ability of a variety of V-complexes to lower elevated glucose levels in diabetic animals treated with V-compounds.[30,61−64] Although only V(IV)-compounds are generally considered in this regard, reports of vanadium in oxidation states V and III are likely to firmly establish the general insulin-like activity of V-compounds.[30,63−68] While insulin-like activity of V-compounds is linked to the inhibitory effects of V-compounds of phosphatases, undoubtedly other activities will emerge now that the vanadium-dependent haloperoxidase and phosphatase association has been made. Indeed, we summarize briefly other enzyme activities that have been reported with V-compounds in the final section of the review. The many discoveries reviewed here document the versatile nature of V-compounds and the multitude of reactions they can catalyze or affect through structural analogy.

The topics in this review have been organized according to oxidation state of the metal in a particular system, since the oxidation state of the vanadium ion is important to the species that direct the chemistry that can occur[16−18,29,31−35] in the reactions. After initially describing the V(V) chemistry, the areas in the biosphere associated with V(V) haloperoxidases and potent inhibition of phosphorylases will be reviewed. This is followed by the V(IV)-containing amavadine and vanabins. The tunicates and the polychaete worm contain both vanadium (IV) and (III) depending on the species. The oxidation state of vanadium in the metal clusters of the nitrogenase is less obvious but generally believed to vary from (II) to (IV). Vanadium in lower oxidation states is not found in the biosphere.[69] In the final section, various other systems are briefly described, and this section is also organized beginning from the highest oxidation state and then sequentially describing systems based on increasing vanadium ion reduction. Since we have reviewed these two different aspects of modeling, this review includes sections targeting more chemically inclined readers as well as sections targeting more biochemically inclined readers, including areas that have not been reviewed previously and others that have frequently been reviewed.

2. Aqueous V(V) Chemistry and the Phosphate−Vanadate Analogy

2.1. Aqueous V(V) Chemistry

Vanadium in oxidation state V forms vanadate and vanadate derivatives in aqueous solution. Vanadate has long been recognized as a structural (**1**−**3**) and electronic analogue of phosphate with similar protonation reactions (eqs 1−3).[9,12−20] This analogy is most evident in the tetrahedral trianionic forms (VO_4^{3-} (**1**) and PO_4^{3-}). However, the similarities of the pK_a values for vanadate (3.5, 7.8, and 12.5)[15] with those of phosphate (2.1, 7.2, and 12.7)[15] document that the analogy also extends to the electronic properties of these species. One difference is that near neutral pH the vanadate monomer exists primarily as a monoanion ($H_2VO_4^-$), while phosphate exists primarily as a dianion (HPO_4^{2-}). In contrast to the high stability of H_3PO_4, the existence of H_3VO_4 although occasionally inferred has not been documented,[70,71] presumably reflecting the conversion to VO_2^+, which is the major species of aqueous vanadate solutions at pH 1. Thermodynamic evidence has now been provided to suggest that the high stability of VO_2^+ is due to the increased coordination number of the hydrated form as compared to H_3VO_4.[72]

$$H_3VO_4 + H_2O \rightleftharpoons H_2VO_4^- + H_3O^+ \quad pK_a\ 3.5 \quad (1)$$

$$H_2VO_4^- + H_2O \rightleftharpoons HVO_4^{2-} + H_3O^+ \quad pK_a\ 7.8 \quad (2)$$

$$HVO_4^{2-} + OH^- \rightleftharpoons VO_4^{3-} + H_2O \quad pK_a\ 12.7 \quad (3)$$

Around neutral pH, $H_2VO_4^-$ and HVO_4^{2-} oligomerize to form dimeric (**4**), tetrameric (**5**), and pentameric

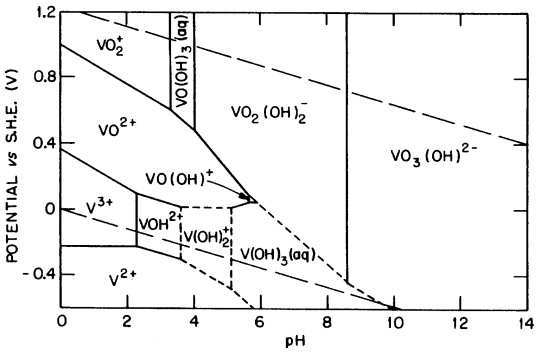
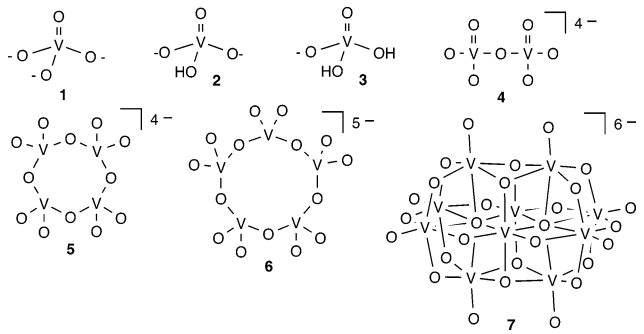


Figure 1. An E vs pH diagram showing the oxidation state of vanadium species as a function of pH and reduction potential. Reproduced with permission from ref 31. Copyright 1976 Wiley & Sons, Inc.

(**6**) structures.[70,71] These species are analogues to pyrophosphate, oligomeric, and polymeric phosphate species and contain anhydride bonds. These vanadate species readily undergo exchange in aqueous solution,[73,74] and since pyrophosphate and the corresponding oligomeric phosphate derivatives are stable and kinetically inert, there is no doubt that the V−P analogy breaks down with regard to the energetics of anhydride bonds and their interconversions. Another important difference lies in the fact that, under physiological conditions, vanadates can undergo redox chemistry[31] while phosphates do not.

Structural information on the oligomeric species in aqueous solution is difficult to obtain. Oligomeric systems have been prepared, primarily from organic solvents[75,76] and in enzyme complexes,[77] and such structural information may not reflect the species formed in aqueous solution. The biological effects of aqueous solutions containing mixtures of oxovanadates reflect that some structural analogy to the corresponding phosphate derivatives does exist. In Figure 1, an E vs pH diagram indicates that at high pH V(V) is the most stable form of vanadium in aqueous solution, whereas at low pH the V(IV) species is more favored.[31] From pH 2 to 6, the major V(V) species is the decamer, $[V_{10}O_{28}]^{6-}$ (**7**) and its various protonated forms; this highly yellow-orange colored species is thermodynamically unstable at neutral and alkaline pH values but, serves as the thermodynamic sink between pH 3 and 6. This oxoanion contains three different types of vanadium atoms, the most unusual being the nonoxo VO_6-type observed for the two central vanadium atoms (see below).



2.2. Mimicking Cellular Metabolites: Vanadate–Phosphate Analogy (Four-Coordinate Vanadium)

Given the central role of phosphate and phosphates in biology, a wide range of effects of two classes of V-compounds, vanadate esters and vanadate anhydrides, can be envisioned. Phosphate esters are common and important cellular metabolites with the phosphate group increasing the solubility and the anionic charge serving to increase the enzyme recognition between cellular substrate and enzyme. In ATP, phosphate serves to store about 7 kcal/mol of energy by forming a anhydride bond compared to the phosphate ester bond storing about 3 kcal/mol. Phosphate plays a key structural and functional role in both the structure and function of RNA and DNA.

The V−P analogy has been used successfully with enzymes that catalyze reactions of phosphate esters (**8**) and phosphate anhydrides (**9**).[14,78−81] Given the importance of the phosphate esters and anhydrides in the literature, the potential formation of vanadium analogues of phosphate esters as shown in eq 4 and phosphate anhydrides as shown in eq 5 have resulted in studies probing both the structural and functional aspects of the corresponding classes of V-compounds.

The effects of vanadate and various derivatives as phosphate analogues will be reviewed in greater detail below describing both the ground state analogy and the transition state analogy with phosphorus compounds. In particular, the analogy of five-coordinate V-compounds with the transition state of phosphate ester hydrolysis has been documented for years. These types of compounds are commonly referred to as transition state analogues of the phosphate ester hydrolysis reactions, and explain why so many V-compounds are known to be potent inhibitors of ribonucleases, phosphatases, ATPases, and other phosphorylases.

$$ROH + H_2VO_4^- \rightleftharpoons ROVO_3H^- + H_2O \quad (4)$$

$$ROPO_3H^- + H_2VO_4^- \rightleftharpoons ROP(O)_2OV(O)_2OH^{2-} + H_2O \quad (5)$$

2.3. Structural Model Studies of Vanadate Esters

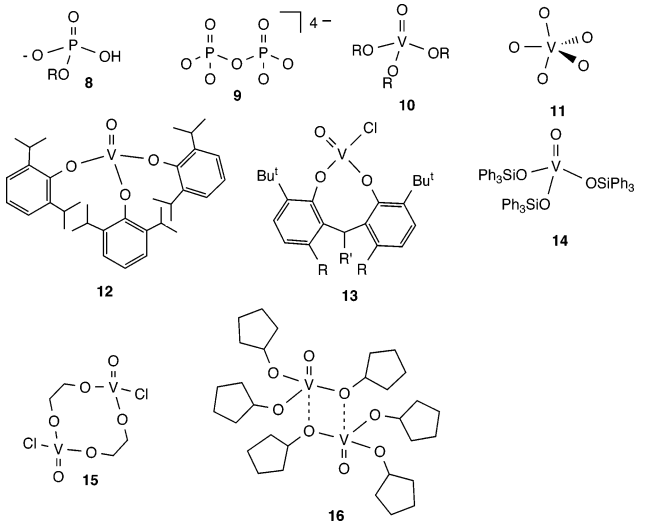
A wide range of model systems have been investigated to examine if trialkoxo oxovanadium(V) complexes (**10**) indeed are structurally similar to triphosphate esters. A large number of vanadium trisalkoxides have been reported,[82−85] with the simplest systems first reported in 1913.[82] In general, the studies focusing on structural characterization have shown that for a wide range of alkoxide ligands, the V(V) center becomes five-coordinate (**11**) by dimerization even in the absence of other coordinating ligands.[85−89] Dimerization is observed in solutions with 10 mM or more of the oxovanadium trisalkoxides and association is observed between molecules.[85,86,89] As the concentration decreases, the fraction of presumed four-coordinate vanadium trisalkoxides increases. Interestingly, the V−P analogy was generally accepted despite an early X-ray structure report of a highly disordered oxovanadium(V)

trimethoxide with a six-coordinate V(V) atom,[90] which highlights differences between these systems. The fact that this simple system fails to show the V−P analogy may be related to the simple alkoxide that allows intersheet interactions between the V and O atoms to generate a wide network with six-coordinate vanadium. Thus, although the most common coordination geometry of trisalkoxide oxovanadium complexes may be five-coordinate, important exceptions occur when the alkoxides used contain steric bulk.

Complexes with various levels of steric bulk have been prepared, and those oxovanadium(V) complexes with *t*-butoxide,[84] *tert*-butylsiloxide,[91] norbornenoxide,[89] adamantanoxide,[89] 2,6-diisopropylphenoxide,[92] or with bidentate, sterically restricted dialkoxides[93,94] contain four-coordinate vanadium. This geometry is observed both in solution[89] and in the solid state.[91−95] Of these examples, one contains "true" four-coordinate vanadium and has recently been structurally characterized as tris(2,6-diisopropylphenoxide)oxovanadium(V) (**12**).[92] This structure firmly establishes that the four-coordinate V−P analogy exists (**8**). This structure is joined by earlier reports with several examples of four-coordinate V(V)-complexes in which the alkoxide/phenoxide/siloxide support the four-coordinate vanadium atom; complexes with a biphenolic diol (**13**),[94] tri-*tert*-butylsiloxide (**14**)[91] and with ethylene diol (**15**) were reported.[93]

A theoretical evaluation of the effects of ligands on vanadium coordination geometry in these simple systems suggested that electronic effects did not induce the coordination differences, but that subtle ligand effects such as the gem-dimethyl effect were invoked.[96] Of the numerous complexes structurally characterized, the system with the cyclopentanol ligand resulted in the vanadium atom showing differential V−O bond lengths (up to about 0.3 Å) (**16**) depending on whether the alkoxide group was directly bound to the vanadium or merely associated with its dinuclear partner.[88] This structure clearly reflects the favorable alkoxide association even with cyclopentanol, a sterically bulky ligand.

Through this work, structural precedence for the formation of vanadate esters of both aliphatic and aromatic alcohols has been demonstrated. The protonation of the vanadium ester has similarly been demonstrated in a model system.[97−99] The five-coordinate geometries of V(V) that serve to mimic transition states of phosphorylase reactions will be described below after the functional aspects of vanadate esters as ground-state analogues have been reviewed.

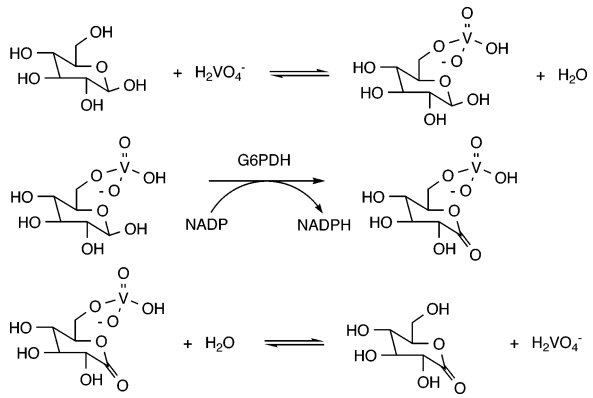


2.4. Vanadate Esters: Functional Analogues of Phosphate Esters

Vanadate esters (alkoxides) form readily in aqueous solution (eq 4) and are found to be enzyme substrates for a range of enzymes including dehydrogenases, isomerases, and aldolases.[14,78−81] The formation constant of the vanadate esters in aqueous solution is on the order of 0.1−0.2 M^{-1} for aliphatic esters[100,101] and is about 3−5 times larger for the formation of aromatic esters.[100,101] When attempting to isolate these vanadate esters, reactions are typically done in organic solutions. The vanadate−ester bonds are much more labile in aqueous solution than phosphate esters.[74] In aqueous solution, these vanadate esters readily fall apart (on a millisecond time scale), and should the alcohol concentration be sufficiently high and the equilibrium be favorable, alcohol exchange will take place.[74] The rate of this reaction and the equilibrium can be fine-tuned; sterically hindered alcohols form alkoxides for which the reactions are much slower.[89] Phosphate esters play an important role in biology, and the potential for forming analogous bonds rapidly at ambient temperature, when phosphate esters only form slowly (solutions take years to equilibrate), is important even though the formation constants of these vanadate esters are small.

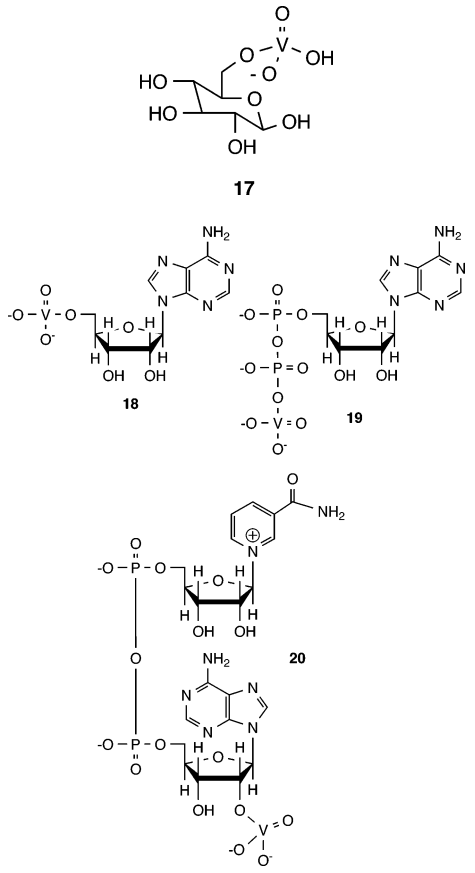
One example of vanadate ester formation is the reaction between D-glucose and vanadate. D-Glucose contains one primary and several secondary hydroxyl groups that can form vanadate esters. Indeed, many species form in such solutions as evidenced by ^{1}H and ^{13}C NMR spectroscopy (data not shown). However, as was shown first by Gresser and Tracey, the small amount of glucose-6-vanadate (**17**) that forms is recognized by glucose-6-phosphate dehydrogenase.[78,79] In Scheme 1, the proposed conversions are

Scheme 1. Reaction of D-Glucose with Vanadate to Form D-Glucose-6-vanadate, a Substrate for Glucose-6-phosphate Dehydrogenase[a]



[a] Refs 78 and 79. The oxidation of D-glucose catalyzed by glucose-6-phosphate dehydrogenase is shown next, followed by the hydrolysis of the vanadate ester.

shown explaining the rapid oxidation of D-glucose to D-gluconate that does not take place in the absence of vanadate at a rapid rate. Practical applications of vanadate esters in enzyme catalyzed synthesis are, in general, better served with arsenate esters[78,79] because the arsenate esters can be formed in higher concentrations than the vanadate esters. Although arsenate esters form more slowly than vanadate esters, incubations with high concentrations of arsenate for several hours will generate correspondingly higher concentrations of arsenate esters. The corresponding solutions containing higher concentrations of vanadate form vanadate oligomers, and barely increase the formation of vanadate monomer, which in turn barely increases the vanadate monomer concentration, resulting in no significant increases in the concentrations of vanadate esters. However, examples of vanadium-cofactor analogues of AMP and NADP have been reported such as AMV (**18**),[102] ADPV (**19**), and NADV (**20**).[80,81] The vanadate ester in NADV is a more effective cofactor in supporting enzyme reactions than the corresponding arsenate ester, which had little, if any, activity.[80,81] The potency of the vanadium analogue has been attributed to the formation of the cyclic derivative for which the enzyme has greater affinity over the natural cofactor NADP.

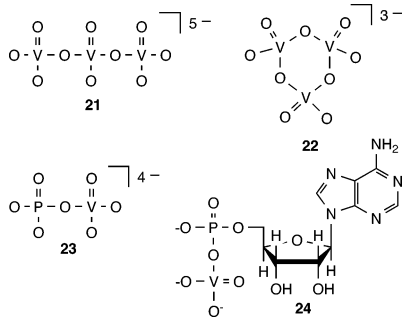


2.5. Vanadate Anhydrides: Structural Analogy with Condensed Phosphates

The analogue to the pyrophosphate bond exists for vanadium in oligomeric vanadate species such as the dimer (**4**), tetramer (**5**), and pentamer (**6**).[70,71] A number of solid-state investigations of the properties of a variety of these V−O materials have been carried out, and go beyond the scope of this work. Suffice to say that the tetramer and pentamer are cyclic in solution and fail to show a structural analogy with the biologically interesting linear phosphates. It has been suggested that the tetramer and the pentamer are observed either because the stability of these cyclic derivatives exceeds that of the linear (**21**) and cyclic (**22**) trimers, or because they may not readily form due to electronic repulsion.[73] The linear trivanadate (**21**) has been observed in solution by ^{51}V NMR spectroscopy from pH about 8.8 to 9.2 at high ionic strength accounting for less than 5% of the total vanadium in solution.[103] The structure for this compound is presumably linear since two different ^{51}V NMR signals are observed for this species. A cyclic trimer has also recently been prepared and structurally characterized[76] as have the known tetra- and pentanuclear analogues.[75,104] However, importantly a linear vanadate trimer was recently reported complexed to phosphatase PhoE from *Bacillus stearothermophilus*.[77] Prior to this report, the structural analogy of these homovanadates were limited to the analogy between pyrophosphate and the vanadate dimer in purely inorganic systems.[105] No information is currently available on systems that support four-coordinate vanadium in a basic O_3POVO_3 unit. However, structural information is available when this unit is part of larger complexes, clusters, or polymeric structures and the vanadium is five- or six-coordinate (vide infra).

Regardless of the missing structural analogue, the potentially most important examples of the vanadate−phosphate systems in biology are the various heterovanadates containing both phosphate and vanadate (**23**). The most important analogues are of ADP, ATP, and other nucleotides. Although a range of ADP and ATP analogues have been prepared in aqueous solution and spectroscopically observed, the most useful analogues are AMPV (**24**) and ADPV (**19**).[106,107] Applications of both these analogues as probes have been met with some success[14] particularly when the analogue is binding in allosteric sites. The limited ability of these analogues to induce the biological responses of the corresponding phosphates presumably can be traced, in part, to the fact that none of these analogues contain the 7 kcal/mol pyrophosphate bond. The corresponding heterovanadate−phosphate bond of 2−3 kcal/mol does not provide the necessary energy. The fact that the vanadate−phosphate analogue does not appear to bind magnesium as do linear phosphates further complicates and diminishes the fit that such an analogue will have when binding to an enzyme. Presumably, the latter explains why fewer effects of vanadate on kinase reactions[17,108] have been reported compared to the well-established inhibition by vanadate of phosphatases.[13]

Although most work in this area has involved vanadium in oxidation state V, both ATP and other nucleotides are expected to form complexes with the vanadyl cation (i.e., vanadium in oxidation state IV). Early studies indeed showed that complexation takes place.[109] More recent studies employing speciation



have extended these studies, and it was concluded that the nucleotide base is involved in complexation of the vanadium.[110,111] The structural information for this type of interaction shows vanadium to have a coordination number greater than four, as described in greater detail below. No information is yet available on the possibility that such complexes can replace ATP or other nucleotide complexes in biology.

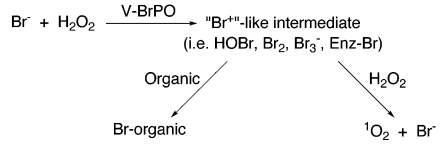
2.6. Potential Future Applications of Vanadium-Containing Ground State Analogues in Enzymology

The vanadium nucleotide analogues, AMV (**18**), AMPV (**24**), and ADPV (**19**), will be reasonable analogues when binding to allosteric sites on proteins and as inhibitors in the active sites of enzymes. The most likely proteins to bind these analogues will have a binding constant of a micromolar or less, and the best binding site will be an allosteric site where Mg^{2+}-binding is not critical for nucleotide binding. Enzymes with less affinity for the parent nucleotides are not likely to be affected by vanadium analogues. D-Glucose-6-phosphate dehydrogenase has also shown high affinity for the vanadate substrate as well as the vanadium-substituted cofactor NADV (**20**). NADV remains the most explored vanadium-containing substrate analogue and its about 20-fold better k_{cat}/K_m provides precedence for applications of vanadium-containing ground-state analogues of organic phosphates. Successful applications of the V−P analogy such as those reported in X-ray crystallographic investigations show very tight binding of the parent anion and the vanadate analogue.[112−127]

3. Haloperoxidases: V(V)-Containing Enzymes and Modeling Studies

Haloperoxidases are enzymes that catalyze the two-electron oxidation of a halide by hydrogen peroxide and consist of chloroperoxidases, bromoperoxidases, and iodoperoxidases. There are three different classes of haloperoxidases known. Two classes contain a prosthetic group, one of which is a heme group in the heme-containing haloperoxidases and the other is a vanadate ion in the case of the vanadium-containing haloperoxidases.[25] In addition, a third class of haloperoxidases, the so-called "metal-free haloperoxidases", was recently described.[21,128] The historical classification and the nomenclature convention are based on the most electronegative halide that the enzyme can oxidize (i.e., the chloroperoxidases (VCPO) can oxidize both Cl^- and Br^- and bromoperoxidases (VBPO) can oxidize Br^-). The reaction is illustrated in Scheme 2.

Scheme 2. Vanadium Bromoperoxidase (VBPO)-Catalyzed Bromide Oxidation by Hydrogen Peroxide[a]



[a] Redrawn from ref 129. Copyright 2001 American Chemical Society.

The discovery of VBPO (and later VCPO), the first enzymes found to use vanadium as a cofactor, has resulted in one of the most active areas in vanadium chemistry and biochemistry. In 1984, bioinorganic chemists were first able to cite an example of an enzyme that required vanadium.[25,130] Should such an enzyme be important and required for existence it would explain how this element could be beneficial at low concentrations. Furthermore, the practical applications of VBPO and VCPO in the halogenation of organic substrates under mild conditions have generated a great deal of interest in industry; the enzyme catalyzes the conversion of X^- to "X^+" (where "X^+" is presumably in the form of HOX, X_2, etc.) followed by nonenzymatic halogenation of the organic substrate (eqs 6 and 7). In the past decade, multiple reviews have been written on various aspects of this area including enzyme structure,[24,30,39−43] function,[9,21−26,30] mechanism,[7,22,23,37] and structural and functional model chemistry.[7,9,29,30,44−48] We refer the readers to these reviews written by contributors in this area for details beyond the scope of this review. Since the discovery of the VHPO enzymes inspired the report of several hundred V(V) and (IV) coordination compounds, many of which have been structurally characterized, but even more that have not been characterized in such detail. We will briefly summarize key recent advances, as well as some of the fundamental experiments important to the studies in this area.

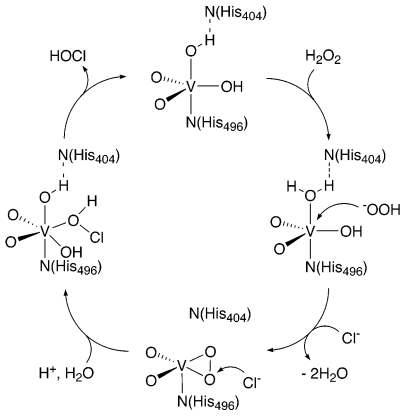
$$Cl^- + H_2O_2 + RH \xrightarrow{VCPO} RCl + 2H_2O \qquad (6)$$

$$Br^- + H_2O_2 + RH \xrightarrow{VBPO} RBr + 2H_2O \qquad (7)$$

3.1. Bromoperoxidase

Although peroxidase activity has been known in marine extracts for many years,[26] it was not until 1984 when Vilter discovered that vanadium content was required in a bromoperoxidase from *Ascophyllum nodosum*.[25,130] The enzyme mechanism was examined using structural, kinetic, and spectroscopic methods, and issues such as the oxidation state of the metal and its coordination geometry have been investigated in detail. The oxidation potentials of the halides are pH-dependent, and, in general, more acidic pH values are required to oxidize the more electronegative halides; this suggests that there is some flexibility in these enzyme systems. Substrate specificity and product distribution remain key areas of interest, and

Scheme 3. Proposed Mechanism for the VCPO-catalyzed Oxidation of Chloride by Hydrogen Peroxide[a]



[a] Refs 7, 139−142, and 146. Reproduced with permission from ref 146. Copyright 2003 Elsevier.

many studies documenting the usefulness of these enzymes in synthesis have been reported.[129,131−133]

The crystal structures of a series of vanadate-dependent haloperoxidases have been solved. There is a significant overlap of the active site between the VBPOs and VCPO despite the fact that these enzymes are distinct from each other. The structures have been solved with several small molecules in the active site, including phosphate or vanadate. The structure of the VCPO from *Curvularia inaequalis*[134,135] was reported first, followed by the structures of the VBPOs from *As. nodosum*.[136] The VBPO residues being phosphorylated are His^{551} in *Corallina officinalis*[39] and His^{486} in *As. nodosum*.[136] The structure of VBPO from *Co. officianalis*[137] followed with the structure of the recombinant bromoperoxidase (r-VBPO) from *Corallina pilulifera*.[138] This enzyme was of particular interest because it contained a second histidine near the active site (His^{478}) instead of the phenylalanine (Phe^{397}) in the VCPO from *C. inaequalis*. Since the structure for VCPO was reported first, much of the structural work with the VBPO was directly compared with that reported for the VCPO,[138] and also with the acid phosphatase (vide infra).[39]

On the basis of spectroscopic evidence, it is now believed that the oxidation state of the vanadium remains at V and does not change during catalysis. A general consensus now exists for the mechanism for both the VBPO and VCPO catalyzed reactions and is described in Scheme 3.[7,139−142] Since the vanadium remains its highest oxidation state, the oxidation of the bromide ion cannot involve reduction of the metal ion in the first step. Mechanistic investigations have involved product analysis, kinetics, and have included studies of both the enzyme and the model systems. The reaction proceeds initially by H_2O_2 addition, which is followed by protonation of the bound peroxide and addition of the halide, respectively. Spectroscopic evidence for the VO_2-O_2 species was obtained using ^{17}O NMR spectroscopy.[143] Enzyme intermediates include a protein−peroxovanadium complex and a protonated protein−peroxovanadium complex. There is no evidence for an intermediate in which the bromide (halide) binds to the vanadium atom.[144,145] The rate-determining step in the catalytic cycle is the nucleophilic attack of the halide on the protonated protein−peroxide complex generating a "Br^+" ("X^+") species, which immediately reacts with organic substrates and brominates (halogenates) them. This step will generate singlet oxygen in the absence of RH, and has been investigated in detail with Cl^-, Br^-, and I^-.

The VBPO and VHPOs in general have little substrate specificity, and this has been used to generate a wide range of products[132] including the bromination and cyclization of terpenes.[131] Specific oxidative conversions of organic substrates in aqueous media are desirable and regio- and/or stereospecific product formation has been achieved to varying degrees. The regiospecific VBPO catalyzed oxidation of 1,3-di-*tert*-butylindole by the marine algae *As. nodosum* and *Co. officinalis* enzymes to produce exclusively nonbrominated 1,3-di-*tert*-butyl-2-indolinone was recently reported.[129] In contrast to most haloperoxidases and perhydrolases, newly detected NADH/FAD-dependent halogenases are substrate-specific and regioselective.[21] These systems do not contain vanadium;[21] however, further information on these enzymes is likely to provide yet more information on another aspect of the vanadium-containing haloperoxidase enzymes.

3.2. Chloroperoxidase

Haloperoxidases have been isolated from red, brown, and green algae,[7,26] seaweed,[7,26] a lichen,[7,147] and a VCPO from terrestrial fungi.[47] In the first X-ray structure of VCPO from the fungus *C. inaequalis*, the vanadium is bound as monoprotonated vanadate(V) in a trigonal−bipyramidal coordination geometry.[134] The metal is coordinated to three oxygens in the equatorial plane, to an OH group at one apical position, and to the Nϵ nitrogen of a histidine at the other apical position. The key residues in the active site are Lys^{353}, Arg^{360}, His^{404}, Arg^{490}, and His^{496} (Figure 2, bottom). The protein fold is mainly α-helical with two four-helix bundles as the main structural motifs. An amino acid sequence comparison with VBPO from the seaweed *As. nodosum* shows high similarities in the metal binding site. All residues interacting with vanadate(V) are conserved except for Lys^{353}, which is an asparagine in VBPO.[134,148] The crystal structure has been determined for the apoenzyme,[135,149] and with vanadate,[134] tungstate,[150] and peroxovanadate[135,149] in the active site. Although overall the two structures of the vanadium- and tungsten-bound proteins are virtually identical, one major difference exists in the active site. In the vanadium−protein structure, the vanadium−histidine bond is strong, whereas in the tungstate−protein bound structure such bonding is either lacking or very weak. This observation demonstrates the fine-tuning that exists in this system for metal−cofactor binding.[150] Investigations into the V-binding in native and mutant chloroperoxidase show that the latter holds the vanadium ion less tightly; however, the mutant maintains bromoperoxidase activity.[151] The X-ray structures of the chloroperoxidase from *C. inaequalis* and active site mutants document the intricate H-bonding network that is in place to

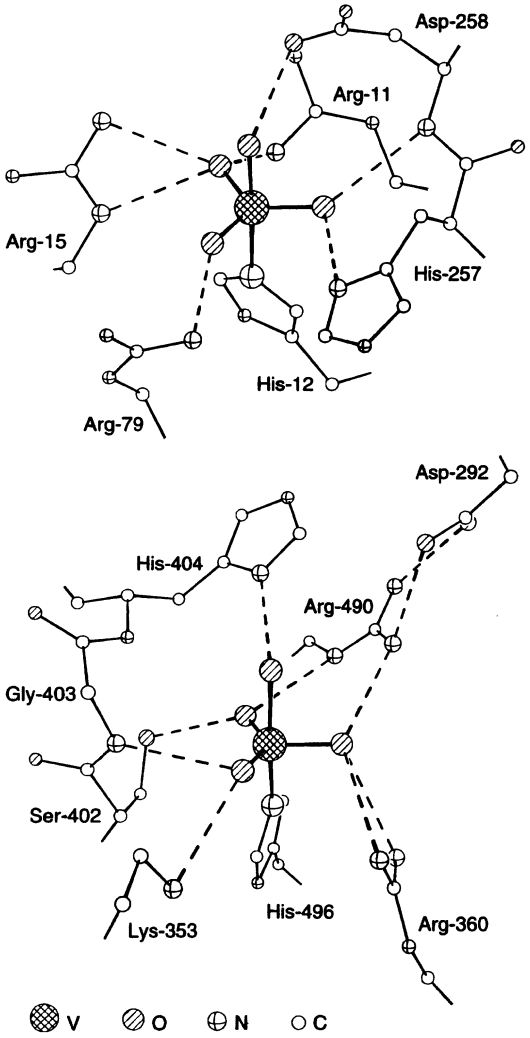


Figure 2. Comparison of the active sites of VCPO and rat prostatic acid phosphatase. Top: Structure of the active site of the rat prostatic acid phosphatase with complexed vanadate.[120] Bottom: Structure of the active site of vanadium haloperoxidase from the fungus *C. inaequalis*.[135] Reprinted with permission from ref 153. Copyright 1999 Wiley-VCH.

stabilize five-coordinate vanadium in the active site.[152]

3.3. From Haloperoxidases to Phosphatases

The amino acid sequence of the active site of VHPOs are conserved within three families of acid phosphatases, including several lipid phosphatases, the mammalian glucose-6-phosphatase, and bacterial nonspecific acid phosphatases.[154−156] These studies were followed by structural confirmation of the similarities between the VCPO and VBPOs[39] and phosphatases.[39,43,154,155,157−159] The amino acids that are involved in phosphate binding in the acid phosphatase enzymes and those that are coordinated to vanadium in the VHPOs appear to be conserved.[39] The structurally characterized VCPO of the fungus *C. inaequalis* shows vanadate covalently linked to the protein through an axially bound histidine residue, His^{496}, embedded in the protein through an extensive hydrogen-bonding network (Figure 2, bottom).[135] In the case of the rat prostatic acid phosphatase, the motif is very similar, although His^{12} is the residue that is bound to vanadate (Figure 2, top).[120]

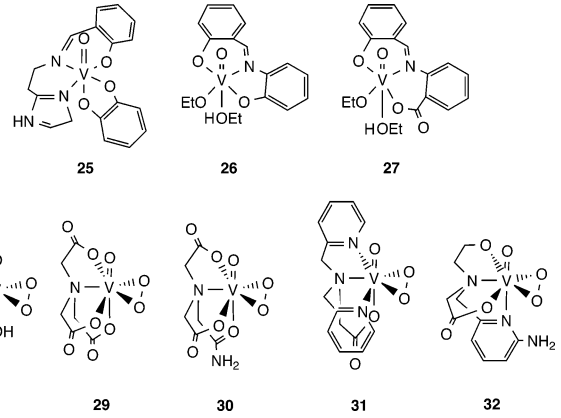
Since phosphatase activity can be catalyzed by apohaloperoxidases,[160,161] the possibility that peroxidase activity could be observed for vanadate-inhibited phosphatases has been investigated for several systems.[39,43,154,156,162,163] The first observation of chloroperoxidase activity was reported with phytase, added vanadate and H_2O_2.[162] Since then reports have been made demonstrating that other phosphatases can be converted to peroxidases in the presence of vanadate and H_2O_2.[39,43,154,156,162,163] Thus, at this time the analogy between these classes of enzymes includes both structural and catalytic aspects. A major issue at the present time (and in the future) is whether the dual activity of these enzymes observed in vitro extends to the function and role of these enzymes in vivo.

It remains to be investigated whether observations with one class of enzymes will lead to reliable predictions with regard to the other class of enzymes, although limited success in this regard can be anticipated. For example, the observation that VCPO has a higher affinity for peroxovanadate than vanadate was used to suggest that glucose-6-phosphatase has a higher affinity for this species considering the evolutionary relationship between the haloperoxidase and glucose-6-phosphatase.[160] This prediction is in contrast to an earlier report in which the K_i value for the dipicolinato monoperoxovanadium(V) complex is about 10-fold less than that of vanadate and the parent dipicolinate acid for alkaline phosphatase.[19] Furthermore, sequence alignment predicted the role of the various amino acid residues based on their role in VCPO and suggested that the His^{404} in VCPO would have the same role as His^{119} in glucose-6-phosphatase. However, the recent studies aiming at identifying the His residue that is covalently bound to the phosphate moiety showed that the His^{176} and not the His^{119} was the residue phosphorylated during catalysis.[164] Another important and different role of residues identified by sequence homology is found for Lys^{76} that in glucose-6-phosphatase was predicted to stabilize substrate by hydrogen bonds as Lys^{353} in VCPO, but instead this residue appears to be found in a membrane bound section of the protein. Although the recognized sequence analogy between VHPO and some phosphatases may not extend to all structural and functional details of the respective protein, such differences are not unexpected considering the differences in reaction mechanism even among the VBPO and VCPO enzymes. The possibility that the haloperoxidase activity is an artifact has also been raised because haloperoxidase activity has also been reported as an artifact of lipases and esterases that normally catalyze hydrolysis of lipids and esters.[128] The dual catalytic activities of the VHPO and phosphatase enzymes are observed, but their importance and possible functions remain to be uncovered.

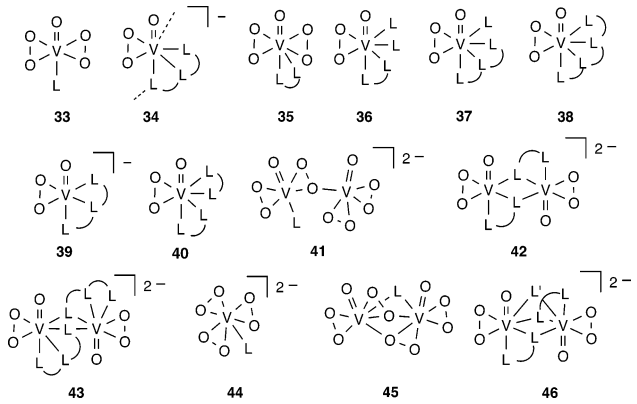
3.4. Structural Modeling Studies of the Vanadium-Dependent Haloperoxidases

Three general categories of complexes have been described as structural model complexes for haloperoxidase enzymes, if structural model complexes

are broadly defined as done in the introductions in the multitude of papers reporting new vanadium coordination complexes for the last two decades. The structural information regarding the haloperoxidases active sites have now shown that most of these complexes are poor structural model systems. The first class defined as containing V(IV) and (V) bound to ligands with O and N donors is the largest and thus contains most coordination complexes. The largest group within this class of compounds are the Schiff base complexes;[165−169] some examples are shown in structures (**25**−**27**).[7] More than 200 crystal structures of V-containing Schiff bases can be found in a search of the crystallographic database and document the widespread interest in these types of compounds. Other fundamental ligand systems such as hydroxyethyliminodiacetate and related simple ligands remain of key interest because of the functional properties of their V-complexes (vide infra). Systems belonging to this class of complexes are shown in structures (**28**−**32**) and are described below with regard to their spectroscopic properties and/or their ability to carry out the VHPO reaction as a functional mimic.[7,9,29,44−48,168,170,171]



The second class of model complexes includes all peroxovanadium complexes. These complexes are of interest not only because they are intermediates in the catalytic cycle of the VHPO reaction, but because of their use as catalysts in organic synthesis.[172,173] In addition, administration of these complexes to cell cultures are found to increase the protein phosphorylation of the insulin receptor (IR) presumably by inhibiting a protein phosphatase (vide infra).[174−177] Aqueous V(V) in the presence of H_2O_2 at acidic pH was found to functionally mimic VBPO[178,179] and a protein−peroxovanadium(V) complex has now been structurally characterized demonstrating the details in the active site when the vanadium ion is coordinated to a peroxo group.[41] A wide range of peroxovanadium complexes have been reported, and several reviews are dedicated to the discussion of the structure and chemistry of these systems.[173,180,181] In Table 1 we summarize the known peroxovanadium complexes with O and N donor atoms and their structures (**33**−**46**).[143,182−229] A summary of all known peroxovanadium(V) systems including those with ligand donors others than O and N have been reported elsewhere.[230] In addition to the spectroscopic characterization of these species, a combined method using electrospray ionization-mass spectrometry (ESI-MS), ^{51}V NMR spectroscopy, and ab initio calculations has been found to be an excellent tool for obtaining direct information about the structure and chemistry of peroxovanadates in solution.[44,231,232]



The number of peroxo groups and the auxiliary ligand that are coordinated to the vanadium atoms dictate the structures (and reactivity) of the peroxovanadium complexes. The peroxo group is bound side-on to the V(V) atom with V−O bond lengths on the order of 1.85 Å and O−O bond lengths of about 1.4 Å. Only one example of a side-on bound alkylperoxide V-complex has been reported.[233] Complexes are known with one, two, three, and four peroxo groups, although only the two former classes of complexes are found under the conditions required of functional mimics. Coordination numbers around the vanadium are generally 6 or 7, and the elaborate dimeric structures often fall apart upon dissolution of the compounds. However, detailed speciation has been characterized for a few simple systems, and this area has been recently reviewed.[234]

The third category of structural model complexes involves complexes in which the H-bonding patterns in the VHPO are the focus of the investigations.[235] At this time, relatively few systems exist in this category of models because the protein structural information to investigate such systems has not been known for that long, and in part because such models require a different approach to designing model systems that contain H-bonded networks.[143,235−237] The first structural characterization of a model complex in which intramolecular H-bonding between a pendant amine functionality and a V(V)-bound peroxide has been reported (Figure 3).[235] This system structurally models the VCPO−$V(O_2)_2$ recently reported.[41] A tungstate layered, double hydroxide catalyst was prepared, inspired by the reaction of the VBPO.[238] The tungstate-exchanged layered double hydroxide halogenates an organic compound, or oxidizes a second H_2O_2 molecule to generate one molecule of excited-state singlet oxygen. The rate of Br^- oxidation is faster than using homogeneous oxometalates or heterogeneous titanium catalysts. Changing the elemental composition of the octahedral layer of the layered double hydroxide can enhance the rate even further.[238] Mimicking the important role of water molecules in the active site has been investigated using model complexes.[144]

Table 1. Summary of the Structural Features of the Crystallographically Characterized Peroxo Model Complexes[a,b,c]

ligand set on V[a]	peroxo ligands	CN	nonperoxo, nonoxo ligand(s)[b]	ligand denticity	structural illustration[c]	ref(s)
N (NO_5)	2	6	ammonia/imidazole	1	**33**	182, 183
NO_2 (NO_5)	1	7[d]	iminodiacetic acid	3	**34**	184
NO (NO_6)	2	7	picolinic acid/3-hydroxy-picolinic acid/ 2,4-pyridine dicarboxylic acid/ 3-acetatoxy-picolinic acid	2	**35**	185, 186
NO_3 (NO_6)	1	7	picolinic acid, $H_2O \times 2$	2,1,1	**36**	187
NO_3 (NO_6)	1	7	dipicolinic acid, H_2O	3,1	**37**	188
NO_3 (NO_6)	1	7	*N*-(2-hydroxyethyl)-iminodiacetic acid	4	**38**	189
N_2O (N_2O_4)	1	6	glycylglycine	3	**39**	190
N_2 (N_2O_5)	2	7	bipyridine (4 structures)	2	**35**	191−194
N_2 (N_2O_5)	2	7	5-nitro-phenanthroline	2	**35**	195
N_2O_2 (N_2O_5)	1		phenanthroline, $H_2O \times 2$	2,1,1	**36**	196
N_2O_2 (N_2O_5)	1	7	picolinic acid × 2/ pyrazine-2-carboxylic acid × 2	2,2	**40**	196, 197
N_2O_2 (N_2O_5)	1	7	D,L-*N*-carboxymethyl-histidine	4	**38**	198
N_2O_2 (N_2O_5)	1	7	ethylenediamine-tetracetic acid (two structures)	4	**38**	199
N_2O_2 (N_2O_5)	1	7	*N*-(carbamoylmethyl)-iminodiacetatic acid (2 structures)/*N*-(carbamoylethyl)-iminodiacetatic acid	4	**38**	200−202
N_2O (N_3O_4)	1	7	1-(2-pyridylazo-)-2-naphthol, pyridine	3,1	**37**	203
N_3O (N_3O_4)	1	7	picolinic acid, bipyridine/picolinic acid, phenanthroline	2,2	**40**	196,204
N_3O (N_3O_4)	1	7	Tris(3,5-diisopropyl-pyrazol-1-yl)borate,H_2O	3,1	**37**	205
N_3O (N_3O_4)	1	7	nitrilotriacetic acid (5 structures)	4	**38**	206−209
N_3O (N_3O_4)	1	7	*N*,*N*-bis(2-pyridylmethyl)-glycine	4	**38**	189
N_3O (N_3O_4)	1	7	*N*,*N*-bis(2-pyridylmethyl)-*β*-alanine	4	**38**	143
N_4 (N_4O_3)	1	7	bipyridine × 2/phenanthroline × 2	2,2	**40**	210
O (O_6)	4	6,6	H_2O (three structures)	1	**41**	211−213
O_2 (O_6)	2	6,6	glycolic acid × 2/D-lactic acid, L-lactic acid (two structures)/L-lactic acid × 2/ mandelic acid × 2	2,2	**42**	214−217
$O_{2,3}$ ($O_{6,7}$)	2	7,7 or 6,6[f]	citric acid (two structures)/ malic acid (four structures)	3,3	**43**	218−221
O (O_7)	3	7	hydroxide	1	**44**	222
O (O_7)	4	7,7	oxide (two structures)/hydroxide	1	**45**	223−225
O_2 (O_7)	2	7	oxalic acid (three structures)	2	**35**	191, 222, 226
O_2 (O_7)	2	7	carbonate	2	**35**	227
O_4 (O_7)	1	7	oxalic acid × 2	2,2	**40**	228
O_5 (O_7)	2	7,7	L-tartaric acid × 2, H_2O (L′)	2,2,1	**46**	229

[a] The complexes are sorted by the overall donor ligand set, which is given in parentheses after the donor ligand set of the nonperoxo and nonoxo ligands. [b] The ligand sets of each structure are separated by a slash. If two (or three) of the same ligands are present in a given structure, then the number of such ligands is denoted by ×2 or ×3; the number of structures of the same complex appears in parentheses. [c] The key to the structural illustrations is shown in Figure 3. [d] The complex forms an extended array with an adjacent V=O group serving as the seventh ligand. [f] The terminal carboxylate oxygens trans to the doubly bonded oxo ligands weakly interact with the vanadium ($V-O_{carboxylate}$ distance ∼ 2.5 Å).

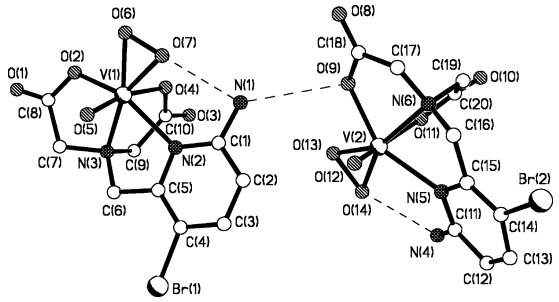


Figure 3. Ball and stick representation of $[VO(O_2)(^{BrNH2}pyg_2)]^-$. All protons have been omitted for clarity. Reproduced from the spatial coordinates given in ref 235. Copyright 2002 American Chemical Society.

3.5. Spectroscopic Modeling Studies of the Vanadium-Dependent Haloperoxidases

^{51}V NMR spectroscopy has become a routine tool for studying V(V)-complexes, and its usefulness and high information content has contributed to the exponential growth in the number of new V(V) coordination complexes. Detailed spectral information on a range of V-complexes has been reported for several decades by the Rehder,[239,240] the Tracey,[14,100,241−243] the Crans[62,67,73,80,81,87,89,95,244,245] groups, and more recently by other groups using this technique to characterize, in detail, the systems that exist in aqueous solution. Several groups have used ^{51}V NMR spectroscopy to (i) predict chemical shifts for known compounds and use ^{51}V NMR as an additional characterization technique and (ii) predict approximate coordination environments for complexes in which characterization is limited.[7,100,244,245] Although caution is needed in such studies, for structurally related systems this method is extremely useful. Such considerations are possible because the ^{51}V chemical shifts can qualitatively be predicted by considering the electronegativity and hardness of the donor atom as well as the specific geometric arrangement of the V-atom. As Pecoraro and co-workers have

demonstrated, such trends do not hold when noninnocent ligands with low energy, ligand-to-metal charge-transfer bands are used in the complexes.[246] For example, catecholate and hydroxamate V(V)-complexes give rise to chemical shifts that are unusually far downfield. However, for complexes with O and N donors such as water, hydroxide, alcohols, monodentate carboxylates, and amines, shifts in the range of −400 to −600 ppm are observed. Shifts upfield of −600 ppm tend to result from negatively charged multidentate ligands that form three- or four-membered chelate rings such as peroxides.[7,239,240]

Early ^{51}V NMR studies on the VBPO from *As. nodosum* show an unusually broad signal at about −1200 ppm and remains an observation that needs further investigation.[247] It is known that association of the quadrupolar vanadium nucleus with a large protein can broaden the signal width if the tumbling of the complex becomes sufficiently slow. Considering the current information of model studies and the crystal structure of the various V-complexes, a protein complex is likely to contain at least three O donors and one N (histidine) donor. Although noninnocent ligand complexes may give rise to a complex with a −1200 ppm chemical shift, the ligands found so far in the crystal structures of protein−V-complexes are not consistent with the reported spectrum.

Pecoraro and co-workers have spearheaded studies using ESEEM on model complexes to explore the coordination environment in the protein system.[7] Recent ESEEM spectra of model complexes suggested that the ESEEM of the reduced enzyme[7] was consistent with the presence of one imidazole ligand in the axial position as well as a second imidazole in the equatorial plane of the vanadyl ion observed in the original ESEEM spectra.[248] The possibility that V(V) binds to both histidine residues in the active site would be consistent with the inactivity of this form of the enzyme because the second histidine that functions as an acid−base catalyst is firmly bound to the protein.[249] ESEEM is also useful for investigation of histidine−V-complexes modeling the histidine−V-complex in proteins.[7,169] Many of the well-characterized model systems are Schiff bases; the corresponding V(V) catechol complex with the ligand HSALIMH is shown (**25**).[250] Recently, Butler and co-workers reported the reactivity of recombinant and mutant vanadium bromoperoxidase from *Co. officinalis*. Mutation of the conserved histidine residue to an alanine (H480A) resulted in the loss of the ability to efficiently oxidize bromide, although the ability to oxidize iodide is retained.[249]

3.6. Functional Modeling of the Vanadium-Dependent Haloperoxidases

Effective modeling of the VHPO activity was obtained for *cis*-dioxovanadium (VO_2^+) in acidic aqueous solution by Butler and co-workers.[178,179] Aqueous V(V) peroxide chemistry is complex, and it was found that the dinuclear species ($V_2O_2(O_2)_3$) was the active species and has been described in detail previously.[7,37,251] These studies importantly demonstrated that not only do systems exist that mimic the enzyme reaction, but if the simple ion can catalyze this reaction, complexing ligands should be able to enhance and/or retard this reaction. Since then, a range of systems have been reported competent to carry out this reaction.[7,9,29,44−48,170,252] These complexes include the first monomeric complexes **26** and **27** reported by Butler and co-workers,[252] which showed that the bromination of the organic substrates proceeded exclusively via an electrophilic mechanism and involved no radical intermediates. Other complexes found to be competent in oxidizing bromide include complexes from additional Schiff base ligands, nitrilotriphosphoric acid and citrate.[189] Interestingly, pyridine-2,6-dicarboxylic acid is found to protect H_2O_2 against reduction by bromide. Other ligands such as picolinic acid do not form sufficiently stable adducts to exist in the presence of H_2O_2.[189]

Pecoraro and co-workers designed a model system with ligands which completed the coordination sphere of an oxoperoxovanadium unit (**28**−**31**).[189,253,254] The most efficient complex of this class, $[VO(O_2)Hheida]^-$ (**28**), showed some properties to be different than those in the Butler systems; acid was required for both catalytic and stoichiometric catalytic activity.[189,253] However, in the presence of excess acid and peroxide, up to 10 turnovers were accomplished within 3 min, and thus increased the rate of reaction by at least an order of magnitude greater than that observed for previous systems. Furthermore, this model system was the first vanadium model compound to demonstrate both the halogenation and catalase reaction catalyzed by VBPO.[254] Detailed mechanistic studies were carried out with these complexes, and supported a mechanism with a protonation preequilibrium preceding the halide oxidation step.[189] Studies with $H_2{}^{18}O_2$ showed persuasively that the oxidation of bromide produced O_2, which was completely labeled with ^{18}O and thus indicated that peroxide is oxidized without oxygen−oxygen bond cleavage.[189]

Pecoraro and co-workers proposed a variation on the enzyme mechansim in which an L group replaces the $EnzO_3$ for the catalysis of the oxidation by model compounds.[7] In this mechanism, the halide is oxidized by peroxovanadium complexes via nucleophilic attack by the halide on a protonated oxoperoxovanadium species. The trihalide is the only product observed under conditions of excess halide and is not directly specified in such a mechanism recognizing that the initial formation of hypohalous acid (HOX) is formally equivalent to $OH^- + X^+$. This variant mechanism is also consistent with the data reported with Butler and co-workers when considering that the proton-independent oxidation reaction in reality results in the liberation of one equivalent of acid when peroxide binds.[7] A two-phase system was designed,[255] and ab initio calculations were carried out to provide evidence for the hypobromite-like V-complex.[256]

3.7. Oxidation of Sulfides by Haloperoxidases (VHPOs)

Application of VBPO, VCPO, and their functional mimics have been explored as potential catalysts in the oxidation of sulfides.[162,257−259] Such activity had

been known for some time with heme-containing haloperoxidases[26] and peroxovanadium compounds.[260] The report that VBPO from the *Co. officinalis* alga catalyze the oxidation of bicyclic sulfides to the corresponding (*S*)-sulfoxides in up to 91% enantiomeric excess accomplished a goal pursued by several groups.[261] VBPO from *As. nodosum*, in contrast, was found to generate the (*R*)-enantiomer of methyl phenyl sulfoxide.[262] Although the r-VCPO from the *C. inaequalis* fungus is competent to oxidize a typical heme peroxidase substrate in contrast to the other VHPOs,[263] the fact that only racemic mixtures of sulfoxides are produced reduces the synthetic potential of this enzyme.[262] Distinct mechanistic differences are proposed between the VCPO and the VBPO to account for the varying product ratios.[264]

The stereoselectivity of the products formed from catalysis by VBPOs is influenced by several factors including pH and the presence of cosolvents.[264,265] Excess hydrogen peroxide was found to reduce both the yield and stereoselectivity of the product formed by the reaction catalyzed by the VBPO from *Co. officinalis*.[261] The addition of bromide ion caused a rapid loss of stereoselectivity presumably due to the chemical oxidation of bromide and subsequent formation of a bromosulfonium intermediate.[264,265] In the reaction catalyzed by the *As. nodosum* VBPO, the presence of electron donating substituents in the para-position of the phenyl ring of methyl phenyl sulfide increased the stereoselectivity of the reaction, while electron-withdrawing substituents decreased the stereoselectivity.[266] Alterations in substrates changed the reaction yields of the enzyme-catalyzed reaction and for the cyano- and nitro-substituted substrates the oxidation reaction was almost completely shut down.

The VBPO from *As. nodosum* catalyzed oxidation reaction using ^{18}O-labeled hydrogen peroxide produced the (*R*)-sulfoxide and is attributed to direct oxygen transfer from the peroxovanadium.[264] In contrast, the r-VCPO from *C. inaequalis* catalyzed formation of a racemic mixture containing small amounts of ^{18}O-labeled sulfoxide. These results suggest differences in the reaction mechanisms for the VBPO- and VCPO-catalyzed reactions. In the heme peroxidase from *Caldariomyces fumago* the sulfoxidation has also been proposed to take place by direct oxygen transfer[267−269] to substrate that is bound near the active site. Similarly the VBPO from *As. nodosum* binds organic substrates,[270] and it is possible that sulfide substrates also undergo direct oxygen transfer.[264] The nature of the protonation state of the peroxovanadium intermediate is still under scrutiny. A mechanism in which the bound peroxide is directly being attacked by the sulfide has been proposed[264] as well as a mechanism in which the peroxide is protonated before sulfide attack.[30,257] Mechanistic details between the VCPO and the VBPO enzymes must vary somewhat to account for the resulting different product ratios.[264] Although the mechanism for the VCPO-catalyzed reaction has been examined in less detail than the VBPO-catalyzed reaction, it has been proposed that the sulfide undergoes a one-electron oxidation to yield a radical cation ($RR'S^{\bullet+}$)[264], which could then go on to abstract oxygen.[267−269] Such a mechanism would be consistent with formation of racemic sulfoxides.

4. Inhibition of Phosphorylases by V-Compounds: Phosphatases, Ribonuclease, Other Phosphorylases, and ATPases

4.1. Phosphatases

Phosphatases catalyze the hydrolysis of phosphate ester bonds (eq 8), and the mechanism involves formation of five-coordinate, high-energy intermediates. These enzymes are generally potently inhibited by vanadate, which is recognized to be a transition state analogue for the phosphatase-catalyzed reaction. The inhibition by oxometalate inhibitors of phosphatases has involved kinetic studies[19,121,174,271−280] as well as structural studies.[112−127]

$$\text{R-PO}_3\text{H}^- + \text{H}_2\text{O} \rightarrow \text{ROH} + \text{H}_2\text{PO}_4^- \qquad (8)$$

Phosphatases can be divided into four classes of enzymes based on the nucleophile in the active site, and three of these classes are illustrated in Figure 4. These enzymes therefore will show some variation in their interaction with the V-compounds.[13,273,281] The first class of phosphatases, the alkaline phosphatase, has a nucleophilic serine residue in the active site so that a hydroxyl group forms the covalent species with the phosphate esters to be hydrolyzed. A second class of phosphatases is comprised of the acid phosphatases and some protein phosphatases; these contain a nitrogen ligand mostly in the form of a histidine residue in the active site, and thus an imidazole group forms the covalent species with the phosphate esters in the active site. Other protein phosphatases have a cysteine residue in the active site so that the thiol group forms the covalent species with the phosphate ester. The final class of phosphatases contains a dinuclear metal ion core in the active site; a metal-bound hydroxyl group is the nucleophile facilitating hydrolysis of the phosphate ester (eq 8). Structural characterization of oxometalate−protein complexes shows the oxometalate in either a four-coordinate[117−120] or five-coordinate geometry[112−116,121] for a range of different phosphatases.

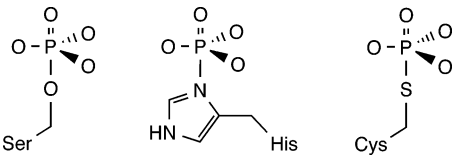


Figure 4. Phosphointermediate forms for an alkaline phosphatase (Ser residue in TS complex), acid phosphatase (His residue in TS complex), and a protein phosphatase (Cys residue in TS complex).

4.1.1. Vanadate: An Imperfect Transition State Analogue of Phosphatases

Phosphatases are, in general, inhibited by oxometalate anions such as vanadate, arsenate, molybdate, and tungstate, which are also often described as phosphate analogues.[12,13,121,273,274,278,279] Studies of less common analogues such as sulfate, rhenate, and

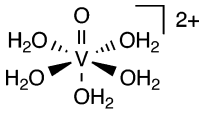
periodate have also been reported.[273,274,280] Although the inhibition of oxometalates should be viewed as product inhibition, the fact that oxovanadates (and a few other oxometalates) also mimic the five-coordinate transition state of phosphate ester hydrolysis (**11**) indicates that the mode of inhibition for these anions is more consistent with the potent transition state analogue inhibition observed for many phosphatases.

The concept of vanadate as a transition state analogue for phosphate ester hydrolysis was first described three decades ago and has now become dogma.[12,13,273,282] The potent inhibition of acid phosphatase by oxometalate anions was attributed to these anions either acting as transition state analogues or by formation of strong chelates.[273] Recent studies, designed to probe how good a transition state analogue vanadate may be, have been probing the origin of the observed inhibition.[283] A perfect transition state analogue has an affinity for the enzyme that exceeds the affinity of the substrate by a factor equivalent to the increased catalytic rate in the enzymatic reaction relative to the corresponding nonenzymatic reaction. Recent studies investigating the affinity of vanadate or vanadate derivatives suggest that although the affinity is enhanced by multiple orders of magnitude, vanadate, for example, comes up short by a few orders of magnitude with regard to its affinity for *Yersinia* PTPase.[283] This result reflects the fact that vanadate is probably not a perfect transition state analogue, although these studies do demonstrate that it comes very close and by many criteria can be characterized as an excellent transition state analogue.

4.1.2. V(IV) Chemistry and Inhibition of Phosphatases by V(IV)

The fact that the $VOSO_4$ has also been reported to be a potent inhibitor for some phosphatases remains an unexplained fact.[275,284] How can a cation and an anion both be potent inhibitors of, for example, *Escherichia coli* alkaline phosphatase? To understand this seeming discrepancy, we will describe the aqueous V(IV) chemistry in detail.

The most well-known V(IV) species is the vanadyl cation (VO^{2+}-cation, $[VO(H_2O)_5]^{2+}$, (**47**)).[12,31−33] The



47

VO^{2+}-cation ($[VO(H_2O)_5]^{2+}$) (**47**) is stable at acidic pH and is the major species at pH 3. This species has a distinct eight-line ambient temperature EPR spectrum, which reflects the 7/2 spin[285] of the vanadium nucleus and is shown in Figure 5. As the pH increases to ~4, a proton begins to be lost (reported VO^{2+} pK_a values range from 5.0 to 6.4[33,285,286]) and the hydrolyzed species $[VO(H_2O)_4(OH)]^+$ forms in solution as shown in Schemes 4 and 5. Scheme 4 shows the relatively simple speciation existing at nano- to submicromolar V(IV) concentrations, and

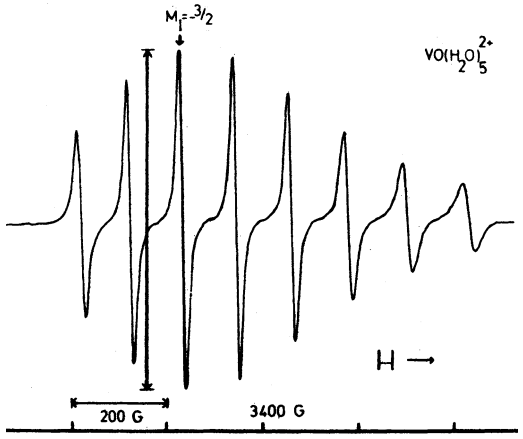
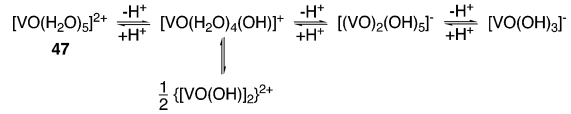


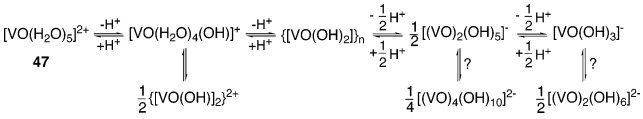
Figure 5. The isotropic, ambient temperature, 8-line EPR spectrum of $[VO(H_2O)_5]^{2+}$. Reprinted with permission from ref 285. Copyright 1975 American Chemical Society.

Scheme 4. Aqueous $[VO(OH_2)_5]^{2+}$ Hydrolysis Products at Nano to Submicromolar V(IV) Concentrations[a]



[a] The insoluble hydroxide $\{VO(OH)_2\}_n$ is in equilibrium with the positively and negatively charged monomeric species.

Scheme 5. Aqueous $[VO(OH_2)_5]^{2+}$ Hydrolysis Products at Micro to Millimolar V(IV) Concentrations



Scheme 5 shows the speciation at millimolar V(IV) concentrations.

As $[VO(H_2O)_4(OH)]^+$ forms, an EPR silent[285] dimer ($[\{VO(OH)\}_2]^{2+}$) begins to form in solutions containing millimolar V(IV) in this pH region.[12,33,230,286,287] Increasing the pH of the solution to above 5 results in an abrupt drop in the EPR signal intensity due to the formation of insoluble $\{VO(OH)_2\}_n$.[285] Upon a further increase in pH, $\{VO(OH)_2\}_n$ dissolves to form a dimer $[(VO)_2(OH)_5]^-$[288] and subsequently, a monomer $[VO(OH)_3]^-$.[289] Only the latter species has been well characterized by means of EPR and UV−Vis spectroscopy,[289] whereas formation of the dimer $[(VO)_2(OH)_5]^-$ was deduced from potentiometric analysis.[288] The prominent existence of $[(VO)_2(OH)_5]^-$ at pH 6−8, although recognized by speciation chemists, has not been generally appreciated by most scientists interested in vanadium chemistry and biochemistry. The description shown here in Figure 6 and Scheme 4 deviates only from those previously reported[12,31,33,230,288] in that a lower concentration range is selected. This seemingly minor modification of the speciation diagram clearly illustrates the existence of negatively charged monomeric V(IV) species at neutral pH and thus provides a very different structural image of the V(IV) species that exist in aqueous solution.

In the neutral pH range (pH 6−8), the concentration of free $[VO(H_2O)_5]^{2+}$ is determined by the {VO-

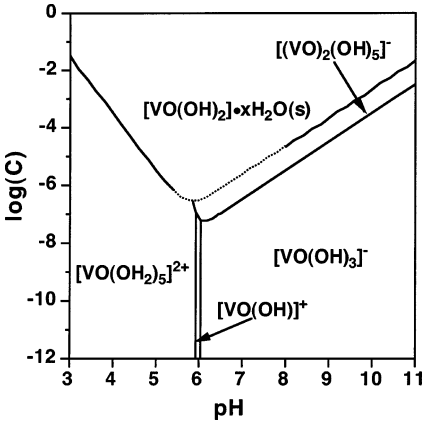


Figure 6. Predominance area diagram for aqueous V(IV) species which is represented as the logarithm of the total molar concentration of vanadium(IV) vs pH. Solid lines represent the condition where the concentrations of two soluble species are equal. The following hydroxo-complexes were assumed $[VO(OH)]^+$ ($\log\beta_{1-1} = -5.94$),[286] $[(VO)_2(OH)_2]^{2+}$ ($\log\beta_{2-2} = -5.94$),[286] $[VO(OH)_3]^-$ ($\log\beta_{1-3} = -18.0$),[33] $[(VO)_2(OH)_5]^-$ ($\log\beta_{2-5} = -22.5$).[288] Here β_{pq} are concentration formation constants $\beta_{pq} = [(VO)_pH_q]^{2p+q}/[VO^{2+}]^p[H^+]^q$ for each species formed according to the general equation $pVO^{2+} + qH^+ = [(VO)_pH_q]^{2p+q}$. $\{VO(OH)_2\}_n$ (s) solubility product value of $K_{sp} = 6.6 \times 10^{-23}$ M^3 (taken from ref 288) was used to evaluate the concentration of free $[VO(OH_2)_5]^{2+}$.

$(OH)_2\}_n$ solubility product unless the total concentration of V(IV) drops below $\sim 10^{-6}$ M. The predicted solubility of V(IV) based solely on the positively charged species ($[VO(H_2O)_5]^{2+}$, $[VO(H_2O)_4(OH)]^+$, and $[\{VO(OH)\}_2]^{2+}$) is significantly less than the observed soluble V(IV). The missing components of V(IV) are a negatively charged monomeric and dimeric anionic species ($[VO(OH)_3]^-$ and $[(VO)_2(OH)_5]^-$). Concentrations of positively charged species under these conditions are extremely low (for example, with a saturated V(IV) solution at pH 7.4 the expected nanomolar concentrations of V(IV) species are calculated to be $[VO(H_2O)_5]^{2+} = 1$, $[VO(H_2O)_4(OH)]^+ = 30$, $[\{VO(OH)\}_2]^{2+} = 0.8$, $[(VO)_2(OH)_5]^- = 350\,000$, and $[VO(OH)_3]^- = 20\,000$), suggesting that indeed negatively charged species play a key role in biological systems under anaerobic noncomplexing conditions.

The formula $[VO(OH)_3]^-$ implies a four-coordinate species, and the spectroscopic signature of this species was investigated using UV−visible and EPR spectroscopy to confirm the geometry.[289] The results were not found to be consistent with four- or five-coordinate species but, rather, with a six-coordinate species. Neither potentiometry nor other spectroscopic methods employed to date can provide information on the exact number of water molecules associated with the metal ion in solution. Presumably, two water molecules are associated with the metal ion making the stoichiometry of this negatively charged ion $[VO(OH)_3(H_2O)_2]^-$. Note that such a stoichiometry would readily convert this anion to the well-known vanadyl cation (**47**) by three protonation steps with no changes in the coordination sphere.

The existence of higher oligomeric species (i.e., $[(VO)_2(OH)_6]^{2-}$, $[(VO)_4(OH)_{10}]^{2-}$, and polymeric $\{(VO)(OH)_3\}_n^{n-}$) was suggested in neutral and basic solution[12,290−292] at submicromolar concentrations. V(IV) concentrations of these species are usually neglected.[293,294] At physiological pH in a noncomplexing media, no EPR spectrum can be observed.[12,285] The observation of a EPR spectrum at neutral pH indicates that either a complexing ligand is present in solution or that the pH is no longer neutral. The presence of buffers and their complexes formed with V(IV) have been used successfully to investigate the complexation of V(IV) to proteins at neutral pH.[12,33,36,230,295]

Recent studies have investigated the interaction of V(IV) with low molecular weight ligands existing in biological systems providing some information on the concentrations of such complexes and the concentration of free V(IV) to be expected. The ligands investigated include phosphate,[296,297] oxalate,[298] lactate,[299] glutathione,[300,301] NAD,[302] NADP,[302] and sugars.[303,304] These analyses were carried out in the presence of a V(IV) complexing ligand and thus attempted to mimic a situation in which a V(IV)-complex was administered to a system. The results support the speciation described above in which $[(VO)_2(OH)_5]^-$ is the predominant species at pH 7−7.5 when the total V(IV) concentration of all species is in the range 1−10 μM.

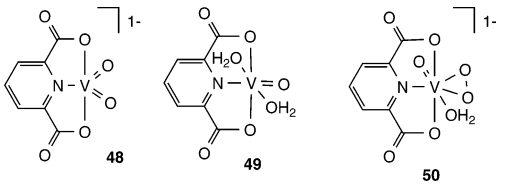
Although the major V(IV) species existing in solution is negatively charged, the complexes formed are between the positively charged V(IV) and negatively charged ligands. This fact reflects that these systems are under thermodynamic control, and the most stable complexes formed will be between negatively charged ligands and VO^{2+} in many biological fluids. To our knowledge, the only stable coordination complexes in which V(IV) is bound to a positively charged ligand occur when V(IV) is bound to a large positively charged protein or peptide. Of all the ligands in the blood plasma (at the level of 10^{-4} M) investigated, citrate[305,306] was the only ligand able to displace strongly bound hydroxo-groups and sequester V(IV).

The complexity of the aqueous V(IV) chemistry and the likelihood of oxidation of V(IV) to V(V) at neutral pH have led most investigators to assume that any affinity of phosphatases for V(IV) reflected the oxidation of V(IV) to V(V). However, when V(IV) is a more potent inhibitor than V(V), this line of reasoning is not sound. As described above, the major monomeric species present in solution at neutral pH is not a positively charged form of V(IV) but $[VO(OH)_3]^-$. The fact that anionic V(IV) species exist near neutral pH would explain the seemingly inconsistent result that V(IV) also inhibits phosphatases[284] despite the fact that the coordination geometry of the ion may be somewhat different than that of phosphorus in phosphate anions. The specific nature of the interaction of VO^{2+} with *E. coli* has not been elucidated;[284] however, potent inhibition is also observed with a wide range of mammalian phosphatases demonstrating that this effect is not limited to the *E. coli* alkaline phosphatase.[275] Inhibition of alkaline phosphatase by aqueous V(IV) may indeed be more similar to the inhibition by vanadate and in line with the inhibition of a transition state analogue.

4.1.3. Inhibition of Alkaline and Acid Phosphatases by Vanadium Compounds

Because the acid and alkaline phosphatases are compatible with V(V)-compounds, they are particularly well suited to test the effects of V-complexes' structures on the phosphatase inhibition. The vanadium model compounds for the phosphatase hydrolysis reaction are five-coordinate complexes that have distorted trigonal−bipyramidal or square−pyramidal geometries (vide supra).[12,29,230] Although the distortion from the perfect trigonal bipyramidal geometry may lessen the structural analogy with the phosphate ester hydrolysis transition state,[307] all V-compounds tested are found to be inhibitors for alkaline and acid phosphatases.[19,174,277,308−313] However, the inhibitory potency of five- (**48**), six- (**49**), and seven-coordinate (**50**) vanadium dipicolinate complexes was tested in detail, and the inhibition was correlated with intact V-compound.[19] The five-coordinate vanadate−dipicolinate complex was the most potent inhibitor, in line with the prediction that a compound with geometry most similar to that of a transition state analogue would be the most potent inhibitor. It is possible that the five-coordinate complex will coordinate to the phosphatase when forming the protein complex and perhaps, in the process, lose a ligand, considering the mechanism of these proteins. Alternatively, the active site may be sufficiently flexible to accommodate a small change. Interestingly, the six- (**49**) and seven-coordinate (**50**) complexes are also strong inhibitors.[19]

Studies with both acid and alkaline phosphatases show the increased affinity of vanadate in the presence of phenol.[274] This observation is interpreted as the formation of the phenyl vanadate ester, which is a much more potent inhibitor for these enzymes than the vanadate anion.[274] These considerations and those introduced below show that a perfect transition state geometry does not seem necessary for potent inhibition of phosphatases and some differences are to be anticipated depending on the specific phosphatase under consideration.



48 **49** **50**

4.1.4. Inhibition of Protein Phosphatases by Vanadium Compounds

Studies carried out with a variety of complexes show that most V-compounds are potent inhibitors of PTPases.[174,277] Lower inhibition is generally observed for the serine/threonine protein phosphatases, which accept serine and threonine phosphate esters as substrates, as compared to the inhibition of tyrosine protein phosphatases.[13] Exceptions have been reported, and there are examples of protein phosphatases other than PTPases that are potently inhibited by vanadate and V-compounds.[278] The effects of a range of different classes of V-compounds have been investigated including V(V)[174,277,313−317] and

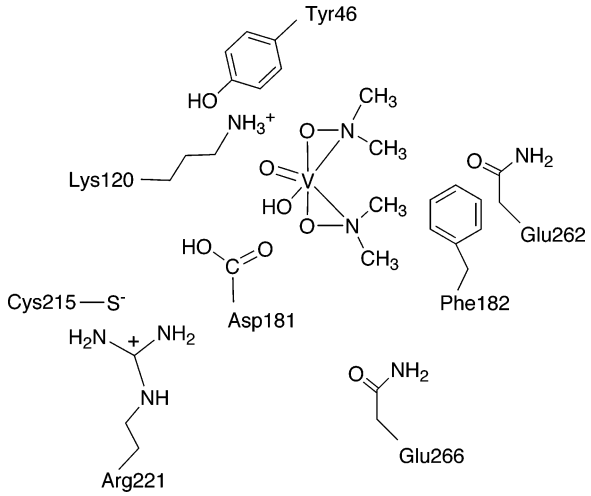


Figure 7. The active site residues in the glutathione-*S*-transferase fusion protein of domain 1 of pp1B obtained by NMR studies and modeling studies with a *N*,*N*-dimethylhydroxylamine derivative placed in the active site. Redrawn from ref 316. Copyright 1998 The Society of Biological Inorganic Chemistry.

V(IV)[318] coordination compounds, peroxovanadium compounds,[174,277,295,314,318,319] hydroxylamidovanadium compounds,[313,315−317] and simple salts.[314,318] Some of these complexes[19] are more potent phosphatase inhibitors than the simple V(V) salt whereas others are less potent.[316] A few V-complexes have no inhibitory activity but may be inhibitors if actually examined under assay conditions where they remain intact.[277] In addition, a variety of studies have been carried out in which the inhibitory effects of these compounds have been measured by indirect means.[13,177,277,318,320−322]

The active site cysteine nucleophile of many protein phosphatases introduces the possibility that redox chemistry can occur between the phosphatase thiol group and a V(V)-compound. Two lines of evidence have been provided that explore this issue. A study was carried out in which the nature of the interaction of vanadate with two protein phosphatases, CD45 and ppB1, was studied using mass spectrometry.[319] Both vanadate and peroxovanadate inhibited these phosphatases, but the mass spectrum of the inhibited protein showed that only peroxovanadate irreversibly modified both protein phosphatases.[319] Since vanadate fails to oxidize these phosphatases, V(IV)-complexes are also not likely to interact reversibly with these proteins. This interpretation was confirmed in studies with pp1B.[318] Kinetic evidence is available for the isoelectronic analogue of the peroxovanadium compounds, the hydroxylamido oxovanadium complexes.[316] Both leucocyte antigen related protein tyrosine phosphatase (LAR) and pp1B were found to be reversibly inhibited by the *N*,*N*-dimethylhydroxylamido V(V)-complex,[316,317] showing a significant difference between how these compounds act and how the corresponding peroxovanadium(V) compounds act. Modeling studies suggest that the R group on the hydroxylamine complex is pointing out of the active site pocket, and explains why structural variation is possible in the R group (Figure 7).[316] Other possible adducts that can form with, for example, thiolate groups[313,315] will also be

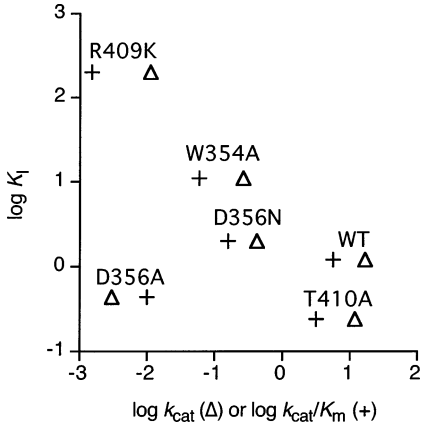


Figure 8. Correlation between k_{cat} and k_{cat}/K_m of vanadate with a series of mutants. Reproduced from ref 283. Copyright 2002 American Chemical Society.

inhibitory. The effects of V-compounds on signaling cascades reflect a synergy between the initiation of signals by PTKs and the loss of control by PTPs and may be attributed to redox-linked activation of key PTKs.[314,323] In addition to the well-known effect of V-compounds as transition state analogues, some V-complexes can also participate in the redox regulation of protein phosphatases.[324]

Projecting from the known redox chemistry of V-compounds, in general, few V-compounds will be able to oxidize the protein phosphatases under physiological conditions. Indeed, reports demonstrate that significant differences are observed when the simple complexes, oxodiperoxo(1,10-phenanthroline)vanadium(V) (pV(phen)) and bis(maltolato)-oxovanadium(IV) [$(VO(malto)_2$] or BMOV), were metabolized after addition to Jurkat cells.[318] The effects of the latter complex are currently of great interest since [$VO(malto)_2$] and the closely related ethyl derivative are currently undergoing evaluation for consideration as therapeutic antidiabetic agents.[325]

The possibility that vanadate was a perfect transition state analogue for *Yersinia* PTPase was investigated using various mutants of the active site (T410A, D356N, W354A, R409K, and D356A).[283] To be a perfect transition state for this PTPase, the affinity for the analogue has to exceed that of the substrate by $\sim 10^{10}$- to 10^{11}-fold, and the apparent dissociation constant for the *Yersinia* is almost 4 orders of magnitude less than this. In addition, the steady-state parameters for wild type and mutants failed to follow the linear trend predicted for a true transition state analogue (Figure 8). Furthermore, the bond orders of vanadate complexes investigated by Raman spectroscopy did not follow the predicted trend. The possibility that phenoxyl vanadate may be a better transition state analogue for the phosphoenzyme and that local structural changes in the active site exist cannot be ruled out and could explain some of the apparent failure to adhere to the predictions.

Studies have been carried out between a small active site pp1B peptide (VHCSAG-NH_2) and V(IV) to model the interactions between pp1B and VO^{2+} (Figure 9). Complexes were observed with the active site cysteine (**51**) and the active site histidine (**52**)

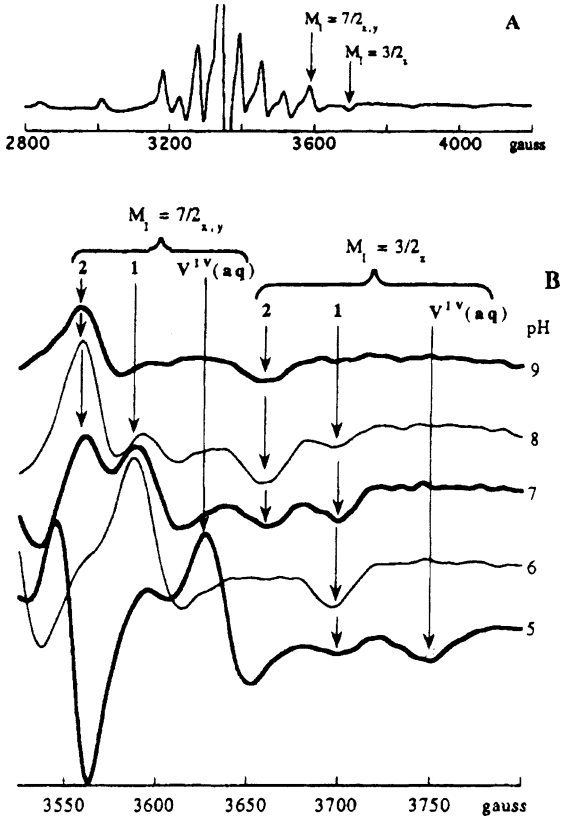
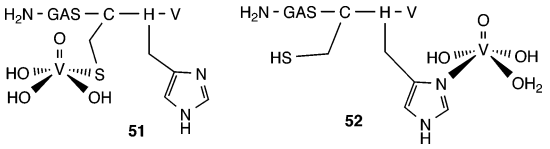


Figure 9. The EPR studies of the complexes formed between the pp1B model peptide (VHCSAG-NH_2) and V(IV). Reprinted with permission from ref 326. Copyright 1995 American Chemical Society.

residues, respectively.[326] The histidine complex is favored at a pH slightly below neutral and the cysteine complex is favored at a pH slightly above neutral. At neutral pH both complexes form (Figure 9). Since this peptide is conserved across the PTP family of proteins, this study documents that both complexes are competent to form[326] and is consistent with the wide range observed for other peptides when complexing to V(IV).[33,300,301]



Inhibition of protein phosphatases are likely to be important to the insulin-like action of V-compounds.[177,318,327−330] In addition, other biological processes that these compounds affect which are likely to involve effects on protein phosphatases include osteoporosis,[312,331−334] apoptosis,[335−341] and cancer.[336,338,342] The specifics with regard to which phosphatase and the level of inhibition required remain to be elucidated.

4.1.5. Inhibition of Purple Acid Phosphatases by Vanadium Compounds

The purple acid phosphatases belong to a family of phosphatases containing a dinuclear metal center, and a metal-bound hydroxide has been invoked as the active site nucleophile in the catalytic mech-

anism.[343−345] Alkaline phosphatase and some acid phosphatases also contain two metal ions, the former containing two zinc(II) ions and the latter containing two Fe, one Fe, and one Zn(II), or one Fe and one Mn(II).[345,346] The purple acid phosphatases, which contain a redox active metal ion, can exhibit yet a different mode of inhibition by vanadate. Uteroferrin is potently inhibited by vanadate, although two types of interactions were observed, one of which describes vanadate as being a slow binding inhibitor.[347] Recently, the initial rates of reaction with a series of oxometalate anions with uteroferrin using stop flow techniques were investigated.[348] Little difference was found among reactions with molybdate, tungstate, and vanadate despite the known differences in lability among these anions. The authors modified the existing mechanism of phosphate ester hydrolysis by suggesting that a step other than the one involving attack on the substituting anion as being rate-limiting.[348] Related oxoanions such as arsenate, molybdate, and tungstate have been investigated in detail using X-ray diffraction, spectroscopic, and enzymatic methods, and have been found to bind in the active site and in some cases bridge the dinuclear metal ions.[343,345] Model studies have been carried out on this system although few included investigations with vanadium.[349] Recently, the ability of the purple acid phosphatase to act as a haloperoxidase has been demonstrated.[350]

Purple acid phosphatase, calceneurin, alkaline phosphatase, and bacteriophage λ protein phosphatase all contain two metal ions important for catalysis. Interestingly, vanadate is a poor inhibitor of calceneurin,[351] but a potent inhibitor of alkaline phosphatase,[274,308] purple acid phosphatase,[347,348] and bacteriophage λ protein phosphatase.[278] These studies demonstrate that the bimetallic phosphatases exhibit a range of responses when exposed to oxometalates.

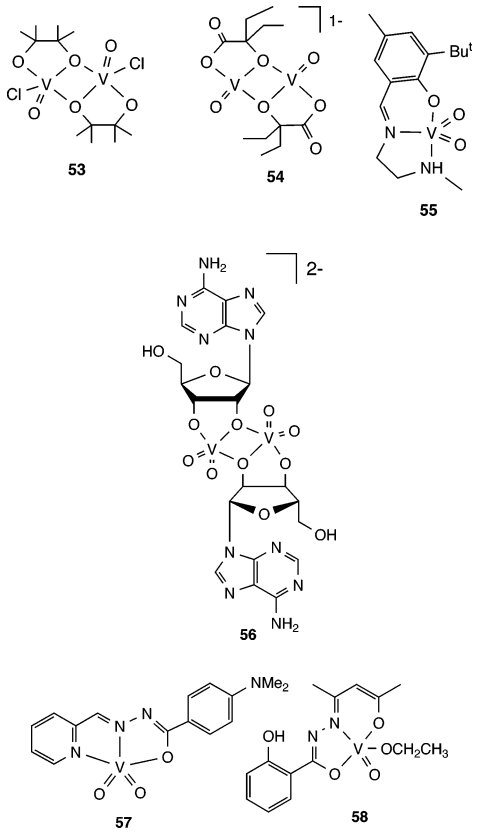
4.1.6. Phosphatase and Other Phosphorylase Inhibition by Oxometalates

Vanadium-containing oxometalates have shown affinity for selected phosphatases such as the *Leishmania* phosphatase.[352] A variety of phosphorylases are inhibited by oxometalates; examples include the rabbit skeletal muscle phosphorylase that has been found to be inhibited by decavanadate, decamolybdate, and decatungstate[353] and ribonuclease by decavanadate.[354]

4.1.7. Structural Models of the Transition State of Phosphate Ester Hydrolysis

Monomeric complexes containing five-coordinate vanadium have been targets of bioinorganic chemists for several years. This is primarily because of the potent inhibitory effects of vanadate on the ribonuclease and phosphatase reactions. Although the first complexes **53**, **54** were dimeric, a variety of monomeric (**15**, **16**, **55**) and dimeric (**56**) complexes have been prepared, and new examples continue to be reported (**12**, **57**, **58**). Although pure trisalkoxides generally dimerize and thus prefer to be five-coordinate (**16**, **53**, **56)**, the use of various types of supporting ligands to prepare and study mononuclear V(V)-complexes have successfully generated several types of complexes (**13**, **14**, **57**, **58)**. The Schiff-base ligands as well as related ligands have been found to be excellent in stabilizing the vanadium atom.[98,99,355−362] In these complexes, the geometries are distorted trigonal−bipyramidal (**55**, **57**)[355,363] and square−pyramidal (**58**).[364] The Schiff-base ligands were also used to structurally characterize and investigate different classes of vanadate ester bonds and the corresponding protonated vanadate ester bonds.[98,99,355−359,361,362,365] The structure, redox properties and reactivity of these complexes are often investigated, and the supporting ligand is found to fine-tune the properties of the O=V−O, O=V−OR, and O=V−O(H)R units.

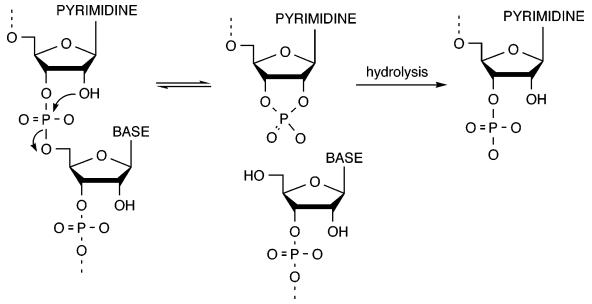
The number of crystallographically characterized pure trisalkoxide parent systems (**12**, **16**, **56**)[89,92,95] is much lower than the number of systems characterized using stabilizing ligands (**13**−**15**, **53**−**55**, **57**, **58**) of which many novel compounds are not specifically shown here.[98,99,355−362,365−367] This testifies as to how critical the supporting ligand is to the properties of these systems and how such a ligand provides structural precedence for biologically relevant chemistry.



4.2. Ribonuclease

Ribonucleases are enzymes that catalyze the cleavage of RNA as shown in a simplified manner in Scheme 6.[368] If the cleavage reaction occurs in the middle of an RNA chain, the ribonuclease is classified as an endonuclease, and if the cleavage occurs at the end of the RNA chain it is classified as an exonuclease. Depending on the type of ribonuclease (A or T_1 for example), the RNA cleavage will be specific for

Scheme 6. The Ribonuclease A Catalyzed Hydrolysis of an RNA Chain Simplified to Two Steps



a pyrimidine or purine base. In the first step of the reaction, a free 5′-hydroxyl group is formed on one RNA chain, and a 2′,3′-cyclic phosphate is formed on the other. In the second step of the reaction, the cyclic phosphate is opened by the reaction with a water molecule generating a 3′-phosphate end on the second RNA chain. Vanadate, vanadyl cation, and their respective complexes potently inhibit hydrolysis of the phosphodiester in RNA and DNA. Vanadium−nucleoside complexes are well-known inhibitors of ribonuclease catalysis having been first reported by Lienhard and co-workers in 1973.[369] The inhibitory V(IV)-nucleoside complex of ribonuclease was first prepared in situ.[370] For a few years, vanadate was the reagent used to prevent ribonuclease cleaving RNA in cellular isolations of RNA until more potent agents were discovered.[369,371,372]

4.2.1. Structural Characterization of Model Compounds for Inhibitors of Ribonuclease

Early structural studies of the protein−V-complex suggested a five-coordinate vanadium atom mimicking the transition state of RNA hydrolysis, Figure 10:[373,374] however, later studies reported an anion bound in the active site as a four-coordinate ground-state analogue.[375] The report of the ground-state vanadate bound in the active site of ribonuclease[375] did not decrease efforts on designing and characterizing model systems of the potent inhibitory V-complex. The investigators recognized the fact that the ribonuclease binding vanadate as a ground-state analogue did not rule out the binding of a transition state analogue to ribonuclease. The most recent vanadate−nucleoside investigation reported contained a five-coordinate vanadate−nucleoside shown in Figure 10.[112]

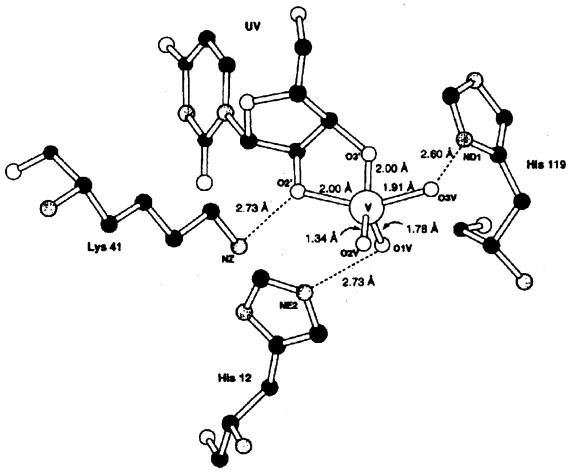


Figure 10. The vanadate−ribonuclease complex showing the five-coordinate vanadate-nucleoside bound in the active site of the protein. Reproduced with permission from ref 112. Copyright 1997 International Union of Crystallography (http://journals.iucr.org/).

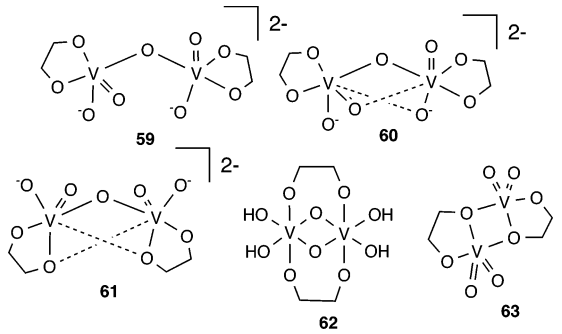
Investigations into the structure of the V-transition state analogue of the ribonuclease reaction have unraveled many of the coordinational preferences of vanadium alkoxides and related compounds in general (see above and below), and should be useful in probing the mechanism of $V_2O_5-P_2O_5$ catalytic conversions of organic substrates. Pinacol was the 1,2-diol used in the first structurally characterized model system and the VO_2 unit was replaced by a VOCl unit (**53**).[87] Another model system that contained a VO_2 unit was used and the diol system was substituted with an α-carboxylic acid (**54**).[376] Several other model systems were also reported showing that mononuclear complexes with five-coordinate V(V) are very stable once a properly designed stabilizing ligand has been used (**55**, **57**, **58**).[355,363,364] In 1995, the problem of disorder in the vanadate−adenosine crystals was overcome, and the X-ray structure of the dinuclear complex was reported (**56**).[377] All the model studies can be summarized as follows: the vanadium atom favors a five-coordinate "transition state" structure and is likely to bind tightly to ribonuclease, with either ribonuclease or an external ligand such as water being the fifth ligand around the vanadium atom.

4.2.2. Characterization of the Nucleoside-Vanadate Complexes that Form in Solution and Inhibit Ribonuclease

Investigations into the structure and formation of the complexes that form in solution between vanadate and nucleosides have continued for more than a decade. The initial studies were carried out using ^{51}V NMR spectroscopy and correctly identified the major species as a 2:2 complex.[106] Due to an initial controversy with regard to identification of the major complexes in solution and the need to determine precisely what the concentration of the 1:1 species was, many studies focused on the speciation of these systems.[102,106,378−381] Identification and quantification of the 1:1 species that was actually bound to ribonuclease was not trivial[379−381] since even under conditions in which this species can be observed, the ^{51}V NMR signal of the 1:1 species overlaps with that of the 2:2 species.

Structural studies characterizing the geometry of the 2:2 species existing in solution were also controversial with several options under consideration (**59**, **60**, **61**, **62**, **63**). Spectroscopic,[307,382,383] theoretical,[96,384] and solid-state model studies[87,355,363,364,376,377] were key in confirming that the vanadium atom was five-coordinate.[307,383] Although this ruled out suggestions of four- and six-coordinate derivatives, three five-coordinate structures remained. The specific structure of the solution complexes were investigated using ^{1}H, ^{13}C, ^{51}V, and ^{17}O NMR spectroscopy,[102,307,377,379−381,383] IR spectroscopy,[307] Raman spec-

troscopy,[307] UV−visible spectroscopy,[307,382] circular dichroism,[382] magnetic circular dichroism,[382] potentiometry,[102,381] and stereochemical considerations.[307,383] By ^{1}H NMR spectroscopy, the major 2:2 uridine complex could be identified in solution (**63**).[307,383] Minor, but structurally similar with respect to ligand coordination, species exist and can undergo exchange to form the one major complex in aqueous solution; in the case of adenosine, the kinetics are less conducive for observation, and low-temperature studies are necessary to reach the analogous conclusion for this system.



4.2.3. Vanadium-Nucleoside Complexes: Functional Inhibitors of Ribonuclease

The concept of transition analogues for this system was recognized early on in a seminal study by Lienhard and co-workers[369] who documented the inhibition of ribonuclease A with a mixture of vanadate and uracil. In these studies, the inhibition was illustrated using both V(IV) and V(V) precursors and thus suggesting that both V(IV)- and V(V)-complexes are potent inhibitors of ribonuclease. Originally the V(IV)-nucleoside inhibitor complex was prepared in situ;[370] however, at neutral pH any free V(IV) will oxidize to V(V), which can form a complex with free nucleosides and generate a second inhibitor complex. Biochemical investigations were undertaken to define the structure of the enzyme and the binding of the vanadium−nucleoside complex.[373,374,385,386] Initial structural studies of the V−protein complex were also reported;[373,374] however, the initial structures were based on a low occupancy of the vanadate−nucleoside adduct in the active site, and coordinates were never deposited in the Protein Data Bank. Recently, the structure of the five-coordinate vanadate−nucleoside complex inside the ribonuclease was elucidated and coordinates are now available.[112,387]

How good a transition state analogue the vanadate−nucleoside complex is depends on whether one expects it to be perfect. If the RNA hydrolysis reaction strictly requires that the transition state have truly trigonal−bipyramidal vanadium geometry, then the vanadium−nucleoside complex will not be a perfect transition state analogue. Indeed, neither the model structures nor the vanadate−adenosine structure (**56**) contains a complex with a vanadium atom in a purely trigonal−bipyramidal geometry. Furthermore, a theoretical investigation examining the electronic properties of V−O and P−O bonds found that dramatic differences exist with regard to bond polarities suggesting that the electronic analogy is not ideal either.[384] From the point of view of inorganic chemistry, one would thus not anticipate vanadate or vanadate derivatives to be perfect transition state analogues.

A reinvestigation of the inhibition constants for binding of the vanadate−uridine complex to ribonuclease A resulted in a submicromolar binding constant[49] that was 100-fold less than originally reported.[369] Using these data to calculate a binding energy, only about 40% of that expected, based on a perfect trigonal−bipyramidal transition state geometry, was obtained.[49] Recently, the potency of the vanadate−nucleoside complex was investigated with several mutants of ribonuclease and these investigators confirmed the conclusion that the vanadium−nucleoside is not a "true and perfect" transition analogue.[50] Although small structural differences existing in the mutants were not considered, which could diminish the observed potency of the vanadate−nucleoside complex as an inhibitor, the fact that vanadate−nucleosides are likely to be potent, but not perfect, transition state analogues is likely to be confirmed. Indeed, considerations regarding what types of phosphorylase reactions V-complexes may be most effective against have been extensively considered.[307,388]

Of the large number of ribonuclease transition state analogues or model compounds reported, most are based on some type of V-complex. Recently, a rhenium-based complex was reported,[389] and documented that other transition metal ions could also be used as transition state analogues. However, these compounds had 10−100 micromolar inhibition constants[389] and thus showed poorer affinity for ribonuclease compared to that of the vanadium−nucleoside complexes. Although the pendulum has swung from describing the vanadium−nucleoside analogue as a potent inhibitor of ribonuclease to a "poor" transition state analogue, it remains to be seen if any other inhibitors can withstand this level of scrutiny. From our point of view any complex that produces half of what would be theoretically expected is a good transition state analogue.

4.3. Other Phosphorylases

Many examples of applications of vanadate as a general inhibitor of phosphorylases other than phosphatases and ribonuclease exist and go beyond the scope of this review. Briefly, the enzymes catalyzing the hydrolysis of a phosphoester bond on a substrate containing one phosphoester bond are formally categorized as phosphomonoesterases and are referred to as phosphatases. When the substrate contains one ester bond and one phosphoranhydride bond, the enzymes are referred to as ribonucleases. For substrates that contain two phosphoester bonds, it is a diphosphoesterase. If the enzyme catalyzes the transfer of a phosphate group from one hydroxyl in a substrate to another hydroxyl group the enzyme is referred to as a phosphomutase. Recent developments will briefly be highlighted here for the enzymes classified as phosphodiesterases and phosphomutases.

Tyrosyl-DNA phosphodiesterase is a member of the phospholipase D superfamily. It functions as a DNA

repair enzyme by hydrolyzing the bond between a tyrosine side chain and a DNA 3′-phosphate.[114,390] This enzyme will facilitate the removal of stalled topoisomerase I-DNA complexes from the DNA strand. Despite the complexity of this enzyme system, when vanadate was used as a substrate, a quaternary complex could be structurally characterized. This phosphodiesterase linked human tyrosyl-DNA phosphodiesterase, a tyrosine-containing peptide, and a single-stranded DNA oligonucleotide into a quaternary complex that mimics the transition state for the first step of the catalytic reaction containing a five-coordinate vanadium.[114,390] The V-complex has a trigonal−bipyramidal geometry, whereas the corresponding tungstate complex shows a six-coordinate metal ion geometry.[114]

The crystal structure of the semi-reduced form of a cyclic nucleotide phosphodiesterase from *Arabidopsis thaliana* has been solved by molecular replacement and refined at a resolution of 1.8 Å.[391] The cyclic nucleotide phosphoesterase converts an ADP-ribose to a 1′,2′-cyclic phosphate. The crystal structure of the native form of this enzyme contains six cysteine residues, four of which are involved in forming two intramolecular disulfide bridges. The bridge between Cys-104 and Cys-110 is opened in the semi-reduced enzyme while the other bridge remains intact; the semi-reduced state of this enzyme was cocrystallized with the inhibitor 2′,3′,-cyclic uridine vanadate. The ligand is bound within the active site, and the mode of binding is in agreement with the previously proposed enzymatic mechanism involving a trigonal−bipyramidal vanadium atom.[391]

The effects of vanadate on phosphoglucomutase have been investigated in detail. Phosphoglucomutase catalyzes the conversion of glucose-6-phosphate to fructose-6-phosphate, and the dephosphoform is potently inhibited by glucose-6-phosphate and glucose-1-phosphate in the presence of vanadate.[392] The inhibition is attributed to the formation of 1-phosphoglucose-6-vanadate and 1-vanadofructose-6-phosphate, which forms a stable complex with the protein. The inhibition constant for 1-phosphoglucose-6-vanadate at pH 7.4 was determined from steady-state kinetic measurements to be 2×10^{-12} M.[393] Calculations of the binding energy of the vanadate complex in phosphoglucomutase revealed an excess of binding energy, over the analogous phosphate derivative, of about 25 kJ/mol and represents about 40% of that expected to be available for a trigonal-bipyramidal transition state.[49]

The effects of vanadate on phosphoglycerate mutase have also been studied. Equilibrium constants for the formation of the two 2,3-bisphosphate analogues were estimated as 2.5 M^{-1} for 2-vanadio-3-phosphoglycerate and 0.2 M^{-1} for 2-phospho-3-vanadioglycerate.[394] The results of the binding study were fully consistent with noncooperative binding of vanadiophosphoglycerate to the two active sites of phosphoglycerate mutase. 2-Vanadio-3-phosphoglycerate was found to bind to the dephospho form of phosphoglycerate mutase with a dissociation constant of approximately 1×10^{-11} M at pH 7 and 7×10^{-11} M at pH 8.[394] The two forms of phosphoglycerate mutase present in mammals, the 2,3-phosphoglycerate-dependent and 2,3-phosphoglycerate-independent forms, show differential affinities for vanadate with the cofactor dependent form responding most potently to vanadate.[395] Cofactor-independent forms of phosphoglycerate mutases are not inhibited by vanadate at the 0.1 mM level, whereas the reversible competitive inhibition yields an apparent K_i value of 15 nM for the cofactor-dependent phosphoglycerate mutase from *E. coli*. The structure of *E. coli* cofactor-dependent phosphoglycerate mutase, complexed with vanadate, has been determined to a resolution of 1.30 Å.[396] Vanadate is bound in the active site, principally as a vanadate trimer and not as monomeric vanadate as reported previously.[120,397] These phosphomutases systems, like those of ribonucleases, provide ample evidence for the applications of vanadate derivatives in enzymology. Some mechanistic investigations in these systems are at a level of detail analyzing the minor differences between a vanadate−protein complex and that of the true transition state complex of phosphoglucomutase.

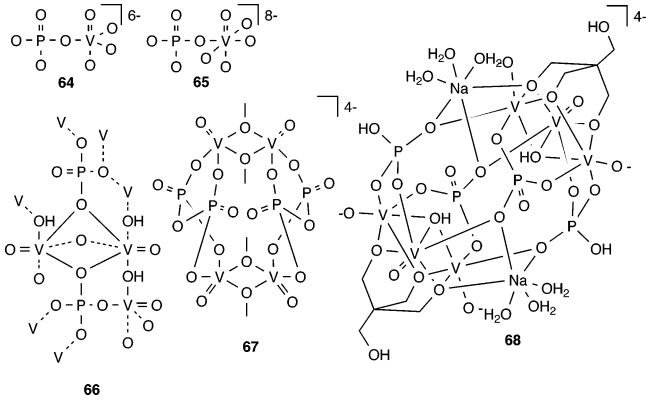
4.4. ATPases

ATPases hydrolyze phosphate−anhydride bonds and have many important roles in biology in cellular energy metabolism. Since there are many different types of ATPases, a wide range of affinities for vanadate can be anticipated and are observed.[2,6,12,60,398,399] The effect of vanadate on some ATPases is very dramatic with a nanomolar inhibition constant having been reported for the Na^+, K^+ ATPases, compared to the millimolar inhibition constants for F_1-ATPases.[398] The ATPase enzymes include many membrane enzymes, and although many details are known with regard to the biochemical mechanisms of some ATPases, inhibitory studies assume that vanadate acts as a phosphate analogue and presumably inhibits the enzymes as a transition state analogue for the phosphoryl group transfer.[400−402] Information on the formation of stable protein−V_i−MgADP complexes similar to the protein−P_i−ADP transition state has been described in detail elsewhere.[60] Often transport inhibition in the presence of vanadate is interpreted as an effect on an ATPase and is used as a test of how a biological system responds to an ATPase inhibitor.[400−402] The reader is referred to the general literature and reviews articles for more information on this topic.[2,6,12,60,399]

4.4.1. Structural Precedence for the Vanadate-Phosphate Anhydride Unit: Five- or Six-Coordinate Vanadium

Heteronuclear adducts of vanadate with phosphate (**22**, **64**, **65**) have been studied extensively and have a greater number of applications in biology as would be anticipated for substrate analogues for ATPases. The vanadate−phosphate or vanadyl−phosphate bond is also of interest in industry since successful catalysts for the large scale preparation of maleic anhydride use V_2O_5 mixed with a phosphorus-oxide catalyst.[403,404] Although few model studies have focused on this unit, structural characterization exists for a range of materials that contain a moiety that can be classified as a vanadate−phosphate anhydride (**22**,

64, **65**). When the vanadium has a coordination number of four (VO_4), the $(O)_3POVO_3$ unit results (**22**). For vanadium with a coordination number of five (VO_5), the $(O)_3POVO_4$ unit results (**64**, **67**).[405−408] If the vanadium has a coordination number of six (VO_6) the $(O)_3POVO_5$ unit results (**66**, **68**).[409−412] An isolated O_3POVO_3 unit (**22**) has not yet been reported; however, it has been observed as part of simple complexes,[408,413,414] larger clusters,[406,407,409,410,415] and polymeric materials.[405,411,416] Many of these materials were prepared by hydrothermal synthesis, but others were prepared using more common synthetic approaches, and a number of these systems have been structurally characterized. Three representative structures are shown (**66**),[411] (**67**),[408] and (**68**)[410] to illustrate the diversity of these complexes, clusters, and polymeric materials in which this type of moiety has been reported.



In the smaller V−P containing complexes, the anhydride unit is supported by an amine ligand[413,414] or a hydroxyl ligand (**67**),[408] and in all these cases the two vanadium atoms are joined through a $\{(OV)_2(\mu\text{−}O)_2\}$ diamond core motif (**53**, **54**), common in dinuclear vanadium alkoxide structures. Significant structural deviations from the bonding for the anticipated simple vanadate−phosphate anhydride system (**22**) are found in all these complexes.[405−412] Since the vanadium engages in additional bonding, the O_3POVO_4 unit (**64**) and the O_3POVO_5 unit (**65**) are those observed. In one case, an amine served to stabilize the polar vanadate−phosphate clusters within a polymer.[416] In general, these structures support a V═O group; however, one example showed a complex with six V−O bonds.[415] These types of non-V═O complexes are rare among high oxidation state V-compounds and are typically seen only in systems with highly stabilizing ligands. In some of the systems, an anhydride linkage is protonated (**66**, **68**). The proton usually resides on an O atom coordinated to two V atoms,[410,411] although two cases were reported with the proton on a phosphate O atom.[412,416]

The fact that the O_3POVO_3 unit was observed in 20 different structures, and the (O)POV(O) unit was observed in 42 structures testifies to the interest in materials containing these types of moieties and their bonding patterns. Interestingly, none of these studies were carried out because of the importance of the vanadate−phosphate anhydride in biology.

4.4.2. Vanadate as a Photocleavage Agent for ATPases

In addition to the biological applications of vanadate to probe ATPases, one area of biomimetic chemistry of great interest to the bioinorganic chemist is the vanadate-induced photolytic cleavage of V-protein complexes. The initial studies were carried out with dynein and myosin, which are ATPases providing energy for intracellular transport and found to cleave at one specific amino acid when exposed to UV light.[7,417−419] This finding was particularly remarkable considering dynein is comprised of two or three heavy chains each of 428 kDa (referred to as α, β, and γ chains), three medium chains at 70−120 kDa, and approximately four light chains of 15−25 kDa to give a total of about 1500 kDa.[420] Since this early discovery, the photolytic cleavage of dynein, myosin, and adenosine kinase has been extensively studied, providing information on the phosphate-vanadate binding site in the presence of free or bound nucleotides.[7,418,419,421]

The oligomeric form of vanadate was found to affect the site of the photocleavage. In the initial study with dynein, photocleavage occurs with a $t_{1/2}$ of about 7 min at the major cleavage site, the V1 site, resulting in fragments of 200 and 228 kDa. Upon closer scrutiny some nonspecific cleavage was found, however, increasing the wavelength from 254 to 365 nm results in cleavage only at the V1 site. Increasing the wavelength to above 400 nm resulted in no observable cleavage since vanadate no longer absorbs light at those wavelengths.[422] The presence of Cr^{2+}, Mn^{2+}, Fe^{2+}, or Co^{2+} also prevents photocleavage and is interpreted to mean that these metal ions bind close to the vanadate binding site.[422] Detailed mechanistic studies have investigated the cleavage products and found the cleaved peptide is in the phosphate binding loop with the sequence $G\text{−}X_1\text{−}X_2\text{−}X_3\text{−}X_4\text{−}G\text{−}K\text{−}G/T$ where X_2 corresponds to the amino acid cleaved.[423,424] Homologous sequences for ATP binding proteins have shown that different amino acids occupy the cleavage site: an alanine in dynein and β ATP synthase, a serine in myosin, a proline in adenylate kinase, and an arginine in α ATP synthase.[417,424−426] Interestingly, it is the location of the amino acid that determines the cleavage and not the nature of the amino acid.

Adding 0.05−0.1 mM vanadate to the dynein heavy chains[427] or the S1 fragment of myosin[421] results in photocleavage at two distinct sites independent of ADP concentration. These sites include the V1 and V2 sites on dynein. The V2 site can also be accessed by binding V_i-Co^{2+}ATP or V_i-Mn^{2+}ATP which quenches cleavage at the V1 site.[427] On the basis of EPR studies showing that in the presence of tris buffer photocleavage is proportional to the development of an EPR signal, it is believed that the process involves formation of a V(IV) species.[421,427] The studies suggested that an oligomeric species is responsible for this reaction, and both a trinuclear[427,428] and tetranuclear V(V) species have been implicated.[421] Since ^{51}V NMR studies have shown that the tetramer binds most strongly to myosin[421] and tetramer is present in higher concentrations in solution, this is the species most likely responsible for this cleavage product. Photocatalyzed cleavage of dynein by Fe(III)

and Rh(III) resulted in different cleavage products,[429] consistent with the increasing number of reports of metal complexes that are competent to cleave peptides.[430]

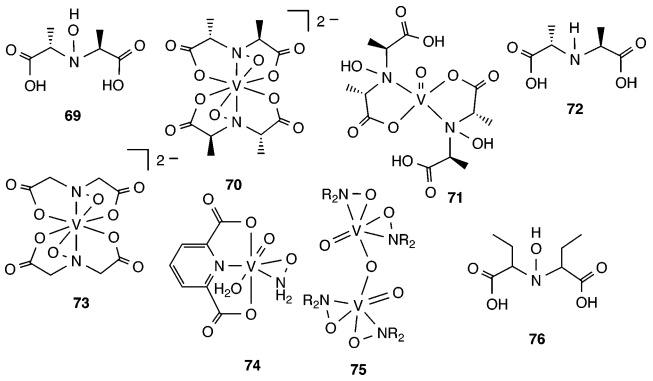
The recent applications of photoinduced cleavage by vanadate/vanadate−nucleotides continue to be very successful in studies of very complex protein systems. Here we describe studies with the F1 ATP synthase that probe the chemical mechanism by which F1 ATP synthases catalyze the synthesis of ATP using vanadate and provide critical information on the conformation at the catalytic site of each β-subunit in the transition state. The MgADP-V-F1 complex was formed in the presence of Mg^{2+}, ADP, vanadate, and protein.[431] Using the photoreactivity of the trigonal-bipyramidal analogue at the γ-P of ATP-site in the presence of UV light and O_2 the MgADP-V-F1 complex was cleaved within the P-loop (GGAGVGKT) of a single β-subunit at Ala^{158}. These results implicate Ala^{158} to be near the γ-P of ATP in the transition state. ADP, although facilitating transition state formation, is not essential for the reaction and cleavage was also observed at Ala^{158}.[432] These findings indicate that Mg^{2+} plays a pivotal role in transition state formation during ATP synthesis catalyzed by ATP synthases, a role that involves both its preferential coordination with phosphate and the repositioning of the P-loop to bring the nonpolar Ala^{158} into the catalytic pocket.[432] Other applications of the vanadate-induced photolytic cleavage include protein systems such as the ATP-binding cassette (ABC) transporter,[433] the human P-glycoprotein,[434] other ATPases,[435] as well as continuing work on myosin.[436−438] The readers are referred to the current literature for more information on this topic.

5. Amavadine and Siderophores

Many types of ligands that exist in nature are capable of sequestering vanadium. Most of these chelating agents, commonly referred to as siderophores, are primarily produced to bind other metal ions, such as iron.[439] Iron bioavailability is controlled by these siderophores which can solubilize environmental iron hydroxides. Siderophores commonly are formed in bacteria, microorganisms, aquatic plants, and yeast, and it is their role to transport metal ions into and out of cells depending on the cellular need. However, such siderophores are not the exclusive chelating agents that are produced in biology and one chelating agent of particular interest is the ligand *S*,*S*-2,2′-hydroxyiminodipropionic acid (H_3hidpa) (**69**) found in the natural product amavadine (**70**), which is produced in some species of the mushroom genus *Amanita*.[7,440]

5.1. Amavadine

Amavadine is a metal complex that contains two equivalents of ligand and one equivalent of vanadium. The H_3hidpa ligand binds V(IV) to form amavadine ($[V(hidpa)_2]^{2-}$) (**70**),[7,440] which is currently the only documented siderophore-like ligand that binds vanadium with greater affinity than any other metal ion ($\log K_2 = 23$).[57] The coordinating functionalities of this ligand are the hydroxylamido and carboxylate groups (**69**). The *Amanita* mushrooms are among the few organisms in the biosphere that accumulate high levels of vanadium (see also the sections on tunicates and the polychaete worm). Common plants and animals that have absorbed vanadium-rich fluids or food will accumulate high levels of vanadium, but these organisms, in contrast to *Amanita* mushrooms and tunicates, do not biosynthesize a vanadium-containing natural product. The recent investigations into molybdenum, niobium, and other metal complexes have shown the amavadine ligand to form complexes with these metal ions; structural and chemical properties similar to the amavadine complex are observed.[441−444] More information on the properties of these complexes is needed to evaluate if the H_3hidpa ligand is specific for vanadium accumulation only, or whether other abundant metal ions such as molybdenum and iron are sequestered by this ligand. There is, however, no doubt that if vanadium is to be effectively accumulated by an organism, a mechanism for chelating V(IV) must be in place. At neutral pH, V(IV) generates a variety of insoluble hydroxides as described above and generally makes the V(IV) unavailable unless a chelating agent solubilizes the metal ion.



5.1.1. Amavadine: Structure

The discovery of high levels of vanadium from the mushroom *Amanita muscaria* was first made in 1931,[445] but it was not until 1972 that the natural product was first isolated by Bayer and Kneifel.[446] Since then studies have taken place with regard to investigating the structure of the natural product,[447−451] determining the product's properties and role,[448−455] identifying new species where this derivative is formed,[456,457] examining its biology,[456,457] and recently, investigating the properties of other metal complexes[441−444] with H_3hidpa (**69**).

The structural elucidation of amavadine was initially controversial because the V(IV) is in an unusual dodecahedral geometry (**70**) which at the time of the isolation of amavadine was unprecedented. Accordingly, in the initial report in which the organic ligand was identified, an alternative structure (**71**) was proposed in which amavadine contained a square-pyramidal oxovanadium(IV) chelated to two λ,λ-*N*-hydroxyimino-α,α′-dipropionic acid (H_3hidpa) moieties (**69**).[446,448] This proposed structure was based on the known coordination chemistry of V(IV), an

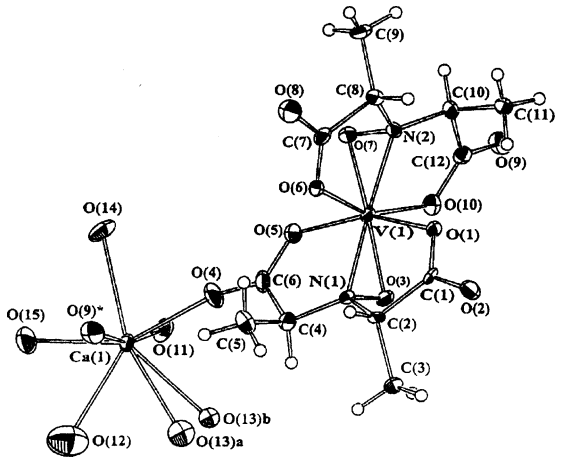


Figure 11. ORTEP view of a portion of the lattice of $[Ca(H_2O)_5][\Delta\text{-}V((S,S)\text{-hidpa})_2]\cdot 2H_2O$. Reproduced with permission from ref 467. Copyright 1999 Wiley-VCH.

axial EPR spectrum consistent with this type of geometry and an absorbance in the IR spectrum at 980 cm^{-1} assigned as a V=O stretching frequency. However, as synthetic model compounds[449−451] and the total synthesis of the natural product were reported,[447] data on this class of compounds began to accumulate which was inconsistent with the initially proposed structure.

Specifically, the much greater formation constant of this complex did not compare well with other square pyramidal V=O containing complexes with aminocarboxylate ligands;[452] amavadine remains one of the more stable V-complexes. Comparing the stability of the V(IV) amavadine complex with V(IV) complexes of iminodipropionic acid (**72**) and *N*-hydroxyiminodipropionic acid (**69**) showed that the NOH group was necessary to achieve this dramatic stability increase. If the NOH group were needed for high stability ($\log \beta_2 = 23$),[448,452,458] it is likely that it would be directly coordinated to the metal ion. Furthermore, the *N*-hydroxyiminodiacetic acid V(IV) complex **73** did not contain the 980 cm^{-1} IR band that was present in the *N*-hydroxyiminodipropionic acid complex; this led to speculation that perhaps no V=O group was present in the natural product. In addition, large-angle X-ray scattering experiments indicated that there were no V−O bond lengths less than 1.9 Å,[448] which might have indicated the presence of a V=O bond (ca. 1.57−1.65 Å). Finally, the Wieghardt[459] (**74**) and Saussine groups[460] (**75**) reported studies with simple hydroxylamine complexes and their proposal of side-on binding of the hydroxylamine functionality to the vanadium was supported by X-ray studies on these simple systems. These facts led Bayer and co-workers to question the validity of their original structural proposal and they revised it in 1987.[448] These studies led to the discovery of a series of V-complexes referred to as nonoxo V-complexes with a variety of different types of ligands.[461−466]

The structural studies were confirmed a few years later when the crystal structures for the model complexes bis(*N*-hydroxyiminodiacetate)vanadate(IV) (**73**)[449,450] and bis(*S,S*-2,2′-hydroxyiminodipropionate)vanadate(IV) (**70**)[451] were reported. Since then two types of amavadine crystals isolated from *Am. muscaria* have been grown in the presence of phosphoric acid and Ca^{2+} ions, respectively (Figure 11).[467] These structures were solved and not only confirmed the proposed structure but also documented the chirality of the amavadine structure both in the neutral and in the anionic form. Amavadine possesses five chiral centers: four chiral carbons all have S configuration and the fifth chiral center is generated by the manner in which the ligands wrap around the vanadium. Spectroscopic studies have shown that as isolated, the natural product contains equal amounts of the Δ and the Λ forms of amavadine.[451] The crystal structure of the vanadium(IV) complex of meso-2,2′-(hydroxyimino)dibutyric acid (**76**) was found to have similar structural features.[58]

5.1.2. Amavadine: Activities and Roles

Amavadine's great hydrolytic stability is accompanied by its reversible one-electron redox properties that have been linked to its possible role in biology as a one-electron redox mediator. The structural properties of the complex are such that very little structural reorganization of the vanadium coordination environment is necessary during the electron-transfer process. However, the one-electron redox couple [V(V)/V(IV)] is very sensitive to solvent. These dramatic solvent differences have been attributed to solvent interaction with some free carboxylate groups and thus explain why with a Pt electrode in water, the potential is +0.53 V (vs SCE) and in dmso the potential drops to +0.03 V (vs SCE).[453] Whether amavadine reacts via an inner or outer sphere mechanism remains to be determined. The original studies, assuming a square−pyramidal complex, proposed an inner-sphere mechanism with the highly exchange-labile axial position in square−pyramidal complexes.[458] Current structural information suggests 0.08, 0.067, and 0.019 Å changes in the V−O (carboxylate), V−O (hydroxylamide), and V−N (hydroxylamide) bond lengths, respectively,[449−451] between the V(IV)- and V(V)-complexes, which are minor changes in complex geometry and are consistent with an outer sphere mechanism. In addition, the complex appears to be hydrolytically intact (vide infra). Accordingly, proposals regarding electron transfer in this system need to be further evaluated.

Although it has been proposed that amavadine serves as an efficient electron-transfer agent in the mushroom, investigations of the reactivity of amavadine have been limited. Studies have shown that amavadine can catalyze thiol oxidation in the absence of hydrogen peroxide (referred to as a peroxidase catalyst by the authors)[468] and in the presence of hydrogen peroxide it can perform peroxidative halogenation, hydroxylation, and oxygenation of alkyl and aromatic substrates.[469] Studies have been done demonstrating that amavadine will electrocatalytically oxidize thiols such as cysteine, glutathione, mercaptoethanol, and a range of related thiols in acidic solution; the catalytic cycle is depicted in Scheme 7.[454] In the electrocatalytic oxidation of thiols, both amavadine and the model complexes show saturation kinetics and thus follow Michaelis−Menton behav-

Scheme 7. The Reaction of Amavadine with Thiols[a]

$$V^{IV} - e^- \rightleftharpoons V^V$$

$$V^V + HSR \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \{V{\bullet}HSR\}$$

$$\{V{\bullet}HSR\} \xrightarrow{k_2} V^{IV} + 1/2\ RSSR + H^+$$

$$(V \equiv [VL_2]^{2-},\ L = HIDA^{3-} \text{ or } HIDPA^{3-})$$

[a] Modified from ref 454.

ior.[454] The initial step in this reaction is the oxidation of the model complex from V(IV) to V(V), followed by the formation of a thiol-V(V) adduct which is the accumulating species. The V(IV) is regenerated by a one-electron oxidation which then releases the oxidized disulfide. Given the coordination environment of the vanadium, the nature of the amavadine−sulfur adduct is not obvious. Should a covalently coordinated complex be required for this reaction, part of the amavadine ligand must dissociate, perhaps only temporarily, for such a reaction to occur. Such a proposal would seem unlikely given the high stability of the complex and its reported inertness in solution. However, the fact that this reaction takes place documents the ability of this complex to convert biologically important substrates and hints at some potential participation in metabolic processes.

Recently, Frausto da Silva, Pombeiro, and coworkers reported that amavadine could convert methane into acetic acid in the presence of a peroxodisulfate salt and trifluoroacetic acid (eq 9).[455] The latter components are necessary for the reaction, whereas the amavadine can be replaced by other V-complexes such as triethanolamine oxovanadium(V) and [VO(malto)$_2$]. In contrast, V(IV)-complexes with ligands such as bicine (*N*,*N*-bis(2-hydroxyethyl)-glycine) and heida (bis(2-hydroxyethyl)*N*,*N*-iminodiacetic acid) in the presence of peroxodisulfate salt and trifluoroacetic acid exhibited much lower reactivity. Using other metal derivatives as well as substituting CF_3COOH with acetonitrile also failed to support the reaction.[455] The authors propose the formation of a methyl radical, which abstracts hydrogen from CH_4 and then undergoes a one-electron oxidation by a V^V-oxo or V^V-peroxo species. Understanding this reaction under such mild conditions will be a major breakthrough and this class of catalyst begs further development.

$$CH_4 \xrightarrow[K_2S_2O_8,HOTf]{\text{vanadium catalyst}} CH_3CO_2H \qquad (9)$$

The Garner group is seeking an increased understanding of the amavadine system by investigating other related metal ion complexes with disubstituted hydroxylimino ligands. As a result, a number of different metal complexes have been reported, of which the titanium,[441,470] molybdenum,[442,443,471] niobium,[444] and tantalum[444] systems will be briefly described here. The titanium system was found to have a structure similar to amavadine; however, allowing a solution of the complex to stand for 2 weeks resulted in changes in the NMR spectrum; this result indicates that solvent molecules do interact with the hydrophilic equatorial surface of the complex.[441] The molybdenum complexes were also found to have a similar structure, but showed more versatile electrochemistry.[442,443] The niobium and tantalum cations yield compounds with similar structures; however, given the large size of these cations, these compounds have several different features. Perhaps the most notable are the quasi-irreversible or irreversible reductions that are observed with these compounds.[444]

5.2. Siderophores

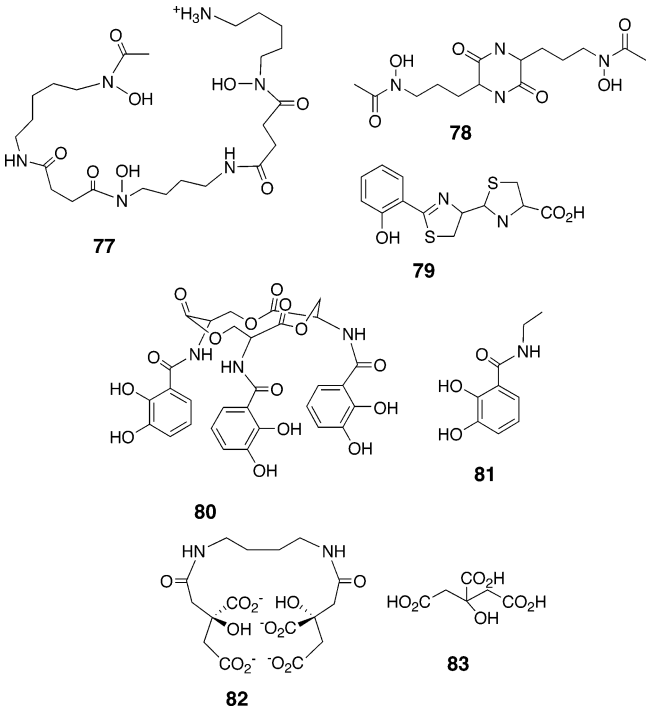
Siderophores provide a number of different coordination environments and a wide range of formation constants with Fe(II) and Fe(III) as well as the accompanying changes in complex lability.[439] The fact that Fe(III) is bound more tightly and that the Fe(II) siderophore complex is more labile can explain how the Fe(III) form can be taken up, the Fe(III)-complex recognized and transported inside the cell where a redox switch is used to release the Fe(II) inside the cell.[439] Despite the obvious biological implications if vanadium is taken up in the place of iron by siderophores, most of the studies have focused on the characterization of new types of V-complexes with siderophores and little quantitative information is available. At this time, the biological role may be explored by examining the effects of V-compounds on siderophore mediated-iron transport and has therefore been described in this review.

5.2.1. The Effects of Vanadium on Siderophore-Mediated Iron Transport

A study was recently reported with *Pseudomonas aeruginosa* showing that $VOSO_4$ inhibits the growth of these Gram-negative bacteria.[472] The effects on Fe-uptake were enhanced in the presence of Fe(III)-chelators. Similar observations were made when examining the iron uptake from desferrioxamine B (**77**) and rhodotorulic acid (**78**) by *Chlorella vulgaris*.[473] Protochelin accumulates in the presence of vanadate in *Azotobacter vinelandii* and vanadate inhibits uptake of ^{55}Fe-protochelin and ^{55}Fe-azotochelin complexes.[474] Pyoverdine-deficient *P. aeruginosa* mutants were more sensitive to $VOSO_4$ than wild-type; however, the addition of pyoverdine to the media did not reverse the effect.[472] The pyochelin (**79**) deficient mutants were more resistant to $VOSO_4$ than wild-type cells, and studies were carried out to link the effects of the V-compound to superoxide formation. Exposure of the cells to paraquat, a compound known to generate a superoxide, increased the resistance to $VOSO_4$ and conversely, exposure to $VOSO_4$ induced resistance to paraquat.[472] The authors suggested that vanadium compromised the utilization of pyoverdine and that the formation of the V-pyochelin can result in Fenton-type reactions and the generation of superoxide.

These results are of interest on several counts. First the interference with Fe transport implies that V can be transported, or at the very least, partially substitute for Fe in siderophore complexes in biology. Since this was observed in several microorganisms and plants, this effect may be general. Second, the observations that one siderophore protected, whereas

the other did not, shows that variable effects can be observed in a biological system. These studies support the expectation that vanadium may affect iron uptake as anticipated based on the chemical similarities of vanadyl cations and iron cations (i.e., their tendencies toward the formation of oligo- and polymeric aqueous ions and their aqueous redox activity).



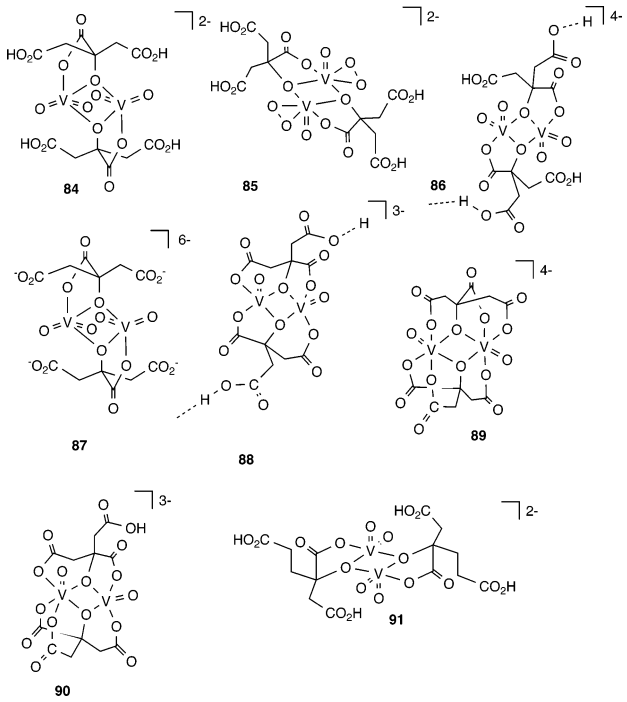
5.2.2. Characterization of Vanadium-Siderophore Complexes and Model Complexes

The crystal structure of the triscatecholamide siderophore, enterobactin (**80**), with V(IV) was determined.[475] In this complex, the catecholate groups were found to be the coordinating functionalities as in the simplest geometrically unrestricted model complex with three *N*-ethyl-3,4-dihydroxybenzamide ligands (**81**). Spectroscopic characterization showed the stability and Δ chirality of the complex originating from the preferential conformation of the triserine backbone.[475] For additional information on catechol chemistry, we refer the reader to the section on tunicate chemistry.

V(IV)- and V(V)-complexes were reported to form between pyoverdine (1:1 stoichiometry) and pyochelin (**79**) (1:1 and 1:2 stoichiometry).[472] Below pH ∼ 1, V(IV) was shown to form a stronger complex with desferrioxamine B (**77**) than iron; however, by pH 4 the formation constant for iron was 7 orders of magnitude greater than that of the vanadyl cation.[476,477] Speciation studies of V(IV) and V(V) with desferrioxamine B (**77**) indicated that at very low pH, nonoxo V(IV)- and (V)-complexes were generated in which the three hydroxamate functional groups occupied the six coordination sites on the vanadium.[478] Upon increasing the pH, the oxo group(s) were restored and the vanadium was complexed by one or two hydroxamate functional groups. Rhizoferrin (**82**), an amino/carboxylate/hydroxy ligand, was shown by UV−vis spectroscopy to bind with both V(V) and V(IV).[479]

5.2.3. Vanadium Citrate Complexes: Structure and Speciation

Citrate (**83**) is known as a siderophore that can complex, in addition to iron, vanadate, and vanadyl ions in aqueous solutions. Citrate is also an important metabolite in mammals where it also serves as a metal chelator. Several bonding modes for V(IV)- and (V)-citrate complexes have been observed (**84**−**90**).[219] The first vanadium−citrate complex, $[V_2O_4(H_2cit)_2]^{2-}$, was structurally characterized in 1995 (**84**). Citrate utilized the hydroxyl and central carboxylate oxygens in the chelate, while the terminal carboxylates remained protonated; only the hydroxylate oxygen atoms bridge the V atoms. Addition of hydrogen peroxide led to the formation of structurally similar diperoxo complexes, $[(VO)_2(O_2)_2(H_2cit)_2]^{2-}$ (**85**).[218,219] By increasing the pH, further deprotonated V(V)-complexes, albeit still with bidentate chelation, have been obtained: $[V_2O_4(Hcit)_2]^{4-}$ (**86**)[480,481] and $[V_2O_4(cit)_2]^{6-}$ (**87**).[482] Typical bond lengths in V(V)-citrate complexes are 1.60−1.64 Å and 1.96−2.02 Å for V═O and V−O, respectively.[218,219,480−484]



The V(IV)-citrate complexes generally have either tri- or tetradentate citrate ligands. An example of a tridentate bonding mode is found in $[V_2O_2(Hcit)(cit)]^{3-}$ (**88**)[485] in which one citrate ligand is monoprotonated while the other is fully deprotonated. For complexes in which the citrate ligands are fully deprotonated, the most common binding mode is tetradentate, which is found for complex anions with the general stoichiometry $[V_2O_2(cit)_2]^{4-}$ (**89**).[482,485−487] A complex with a mixed tridentate/tetradentate bonding mode, albeit with 2:2 stoichiometry (**88**),[485] has also been isolated (**90**).[488] Typical bond lengths in V(IV)-citrate complexes are 1.58−1.61 and 1.95−2.23 Å for V═O and V−O, respectively.[482,485−488] The predominant

features in all of these 2:2 complexes is the diamond-core [V−O−V−O] motif predominant in V(V) alkoxide chemistry. The V(V)-citrate complexes tend to be five-coordinate, with the exception of the six-coordinate peroxo citrate complexes, while the V(IV)-complexes are generally six-coordinate. Changes in pH did not significantly affect the structure of the complex ion, aside from the protonation state(s) of the terminal carboxylates, in either the V(V)[480]- or V(IV)[485]-citrate complexes. However, some of these complexes were found to convert to each other in solution when the pH was changed.[480]

Aqueous speciation studies of the vanadate(V)−citrate found primarily 2:1 complexes.[489] In light of the structural results and the closeness of the fit, one species, assumed to be a 1:1 complex, may be a 2:2 species.[489] The vanadyl(IV)−citrate speciation system indicated the presence of primarily 2:2 complexes, although below pH 4.5 1:1 species were detected while 1:2 (metal:ligand) species could be detected above pH 8.

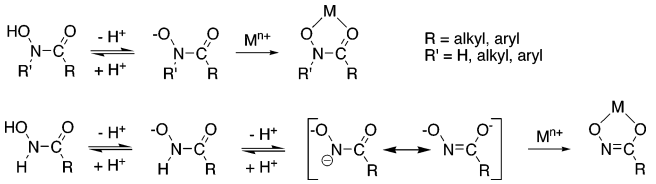
A close relative of citrate, homocitrate, is a component of the cofactor in the nitrogenases (Nases), including V-nitrogenase (vide infra). Only one structure has been crystallographically determined for a V(V)-complex with homocitrate (**91**).[490] Discussion of Nase and model compounds is presented below in the section on low-valent vanadium chemistry.

5.2.4. V(V) and V(IV) Hydroxamate Complexes

Hydroxamic acids have the general formula RC(=O)N(R′)OH, and their deprotonation and binding modes are depicted in Scheme 8. Hydroxamates readily deprotonate to monoanions followed by binding to the metal ion in a bidentate fashion through the deprotonated hydroxy oxygen and the neutral carbonyl oxygen atoms, respectively. When R′ is H, two deprotonation steps result in a dianionic ligand which has two resonance forms. This anion is generally referred to as a hydroximate due to the C=N resonance form.[491] Hydroxamate complexes have been widely used in spectrophotometric determinations of vanadium due to the intense color of the resulting complexes.[492,493] Desferrioxamine B (**77**), an abundant hydroxamate-containing siderophore that forms complexes with both V(IV) and V(V),[478,494] and has sparked renewed interest in the study of vanadium−hydroxamate interactions.

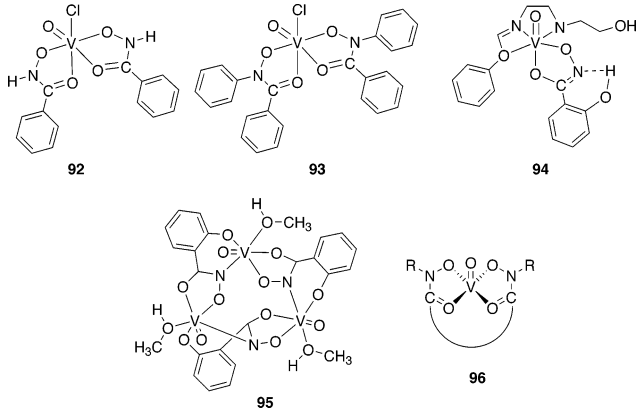
V(V) hydroxamates are far more common due to the tendency of V(III) and V(IV)-complexes to abstract oxygen from hydroxamic acids.[495] At submillimolar concentrations, aryl hydroxamates form 1:1 five- or six-coordinate complexes at pH 7.5 as determined by ^{51}V NMR spectroscopy; at higher concentrations the 1:2 complex predominates.[496] For the 1:1

Scheme 8. Deprotonation Products and Binding Modes of Hydroxamates



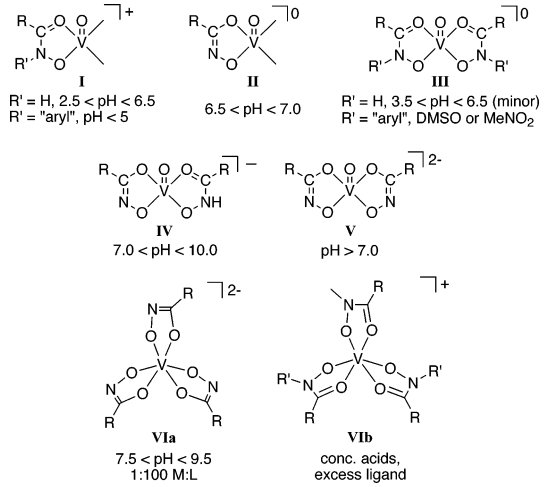
complexes, ^{15}N NMR spectra suggest the hydroxamate to be monoanionic and ^{13}C NMR spectra indicate that the carbonyl oxygen is weakly coordinated. The log K_f values obtained (ca. 1−4)[496] were much lower than those obtained in more acidic solutions.[497,498] Speciation studies on alkylhydroxamic acid−vanadate complexes give log K_f values on the order of 7−38 depending on the pH.[499,500] Cinnamoylhydroxamate was shown to form 1:1 and 1:2 complexes with V(V) at 1.8 M HCl in 2-methyl-4-pentanone; the stepwise formation constants were given and an octahedral coordination geometry was suggested.[501]

The first structurally characterized V-hydroxamate complexes were the V(V)-complexes of benzohydroxamic acid (**92**),[502] the dihydroxamate *N*,*N*′-dihydroxy-*N*,*N*′-diisopropylheptanediamide,[502] and *N*-phenylbenzohydroxamic acid (**93**).[503] All the complexes are six-coordinate and the ligands are monoanionic; the benzohydroxamate and *N*-phenylbenzohydroxamate complexes are monomeric, while the dihydroxamate ligand yielded a 2:2 complex. A series of salicylhydroxamate V(V)-complexes with tridentate ternary ligands were prepared; the derivative with *N*-(salicylideneaminato)-*N*′-(2-hydroxyethyl)ethylenediamine was crystallized (**94**).[246] This complex and its congeners contain a dianionic salicylhydroxamate ligand (i.e., a hydroximate), and a strong hydrogen bond was observed between the phenolic hydroxyl group and the deprotonated imino nitrogen.[246] A trinuclear vanadium(V) complex, $[VO(shi)(OCH_3)]_3$ (**95**), was prepared from $VO(acac)_2$ or VCl_3, salicylhydroxamic acid, and $NaOCH_3$ in methanol and structurally characterized. Each trianionic ligand is tetradentate with two donor atoms bound to each vanadium center to generate a metallocrown structure.[504] Similar metallocrown structures have also been prepared from modified salicylhydroxamic acids.[505] The first mixed hydroxamate/hydrazone V(V)-complex contained a monoanionic salicylhydroxamate ligand.[506] Other structurally characterized V(V) ternary complexes include 4-(2-(salicylideneamino)-ethyl)-imidazole/salicylhydroximate,[250] benzohydroxamic acids/*N*-salicylideneglycine and *N*-(2-carboxyphenyl)salicylideneamine,[507] and *N*-phenylbenzohydroxamic acid/acetylacetone benzoylhydrazonato and salicylidene-L-alaninato.[508]



To date, no crystallographic data for V(IV) hydroxamate complexes are available, although solution studies indicate that V(IV) hydroxamate complexes

Chart 1. Hydroxamate and Pseudohydroxamate Vanadyl Complexes



are present in some solutions.[478,491,494] This is likely due to the instability of the hydroxamate ligand which can be readily reduced via oxygen abstraction by V(IV) or V(III).[495] Nonetheless, in solution desferrioxamine B (**77**) has been shown to complex with both V(IV)[478,494] and V(V).[478] EPR studies show the formation of nonoxo complexes of these metal ions at low pH; when the pH is gradually increased the oxo ligands are restored. Stability constants (log β) for the V(IV)-desferrioxamine were on the order of 30−40.[478] A rhodotorulic acid (**78**) V(IV)-complex has been prepared both as the vanadyl complex and as a nonoxo complex; elemental analysis of the nonoxo complex, however, showed a ratio of 3:2.2 (metal: ligand).[502] Reversible V^{4+}/V^{3+} reductions were observed for both the vanadyl and nonoxo V(IV)-complexes.[502]

A series of hydroxamate and pseudohydroxamate vanadyl complexes were prepared, and EPR spectroscopy was used to conduct a speciation study (Chart 1).[491] For the aceto- and benzohydroxamic acid complexes a variety of species were observed. At acidic pH, 1:1 complexes predominate with a neutral 1:2 species present in minor concentrations. In basic conditions, 1:2 complexes are found and at 100-fold excess ligand, the nonoxo trihydroxamate complex is observed between pH 7.5 and 9.5. The *N*-phenylbenzohydroxamic acid and 2-hydroxypyridine-*N*-oxide, which only have one ionizable proton, form the 1:1 and 1:2 species, respectively. In very acidic conditions with excess ligand, a trihydroxamate complex is formed.[491]

Biological studies focusing on applications of hydroxamate V-complexes encompass a variety of areas. A number of dihydroxamate complexes of V(IV) and V(V) were found to stimulate glucose metabolism in rat adipocytes.[509] Interestingly, the hydrophobic dihydroxamate ligand complexes were more effective at stimulating glucose metabolism, and it was suggested that the hydroxamates are acting like ionophores by facilitating vanadium transport into the cells.[509] ESEEM spectroscopic was undertaken to examine the coordination environment in Shechter's complexes, and the hydroxamates were found to have a cis orientation and to occupy a plane roughly perpendicular (within 20°) to the V=O axis (**96**).[510] V(IV)- and (V)-complexes of glutamine- and aspartate-derivatized hydroxamate ligands have also been found to exhibit insulin-enhancing activity and activate lipogenesis.[328,511] A distamycin-linked dihydroxamate complex of V(IV) in the presence of hydrogen peroxide was shown to selectively cleave DNA depending on the length of the linker group and the AT sequences.[512] Aryl and alkyl hydroxamate V(V)-complexes were found to be inhibitors of the class C β-lactamase in *Enterobacter cloacae* P99.[513,514] For the β-lactamase, the largest K_i value obtained was 0.48 mM; the association/dissociation rate constants and other studies implicate strong binding of the V-complex in the active site.[513,514] Other enzymes inhibited by vanadate−hydroxamate complexes include serine amidohydrolase, chymotrypsin, and elastase.[514]

Synthetic applications for vanadium−hydroxamates have extended this area to metal extraction processes and asymmetric epoxidations.[515−518] The possibility of diastereomeric intermediate formation may limit the achievable enantiomeric purity.[518]

6. Tunicates and the Polychaete Fan Worm

Although high concentrations of vanadium have often been found in various tissues or organs of living organisms, few organisms in the animal kingdom are actually known to accumulate vanadium. Recently, it has been reported that in addition to the high levels of vanadium in ascidians,[4,7] the fan worm *Pseudopotamilla occelata* also accumulates vanadium.[519−521]

6.1. Tunicates

Tunicates (ascidians or sea squirts) are invertebrate marine organisms and, depending on the species, accumulate vanadium in their blood. The V-containing species were first discovered in 1911 by Henze,[4,7,522] and since then, bioinorganic chemists and biological scientists have been interested in these animals that can concentrate vanadium from seawater ($\sim 10^{-8}$ M) in various cells of the tunicate (Table 2). Bioinorganic chemists have investigated the form and storage of vanadium in these organisms, its redox conversion from V(V) in seawater to V(IV) and V(III), and also how and why vanadium is accumulated. Although much is known about most of

Table 2. Concentration of Vanadium (μM) in Ascidian Tissues[a]

species	tunic	mantle	branchial	serum	blood cells
Phlebobranchia					
Ascidia gemmata	ND	ND	ND	ND	347 200
A. ahodori	2400	11 200	12 900	1000	59 900
A. sydneiensis	60	700	1.400	50	12 800
Phallusia mammillata	30	900	2.900	ND	19 300
Ciona intestinalis	3	700	700	8	600
Stolidobranchia					
Styela plicata	5	1	1	3	3
Halocynthia roretzi	10	1	4	1	7
H. aurantium	2	2	2	ND	4

[a] Data taken from ref 10. [b] ND, not determined.

these topics,[3−7,9,16,59,523] the function of vanadium in tunicates remains elusive even though many credible hypotheses have been put forth. Other aspects of these animals, including their biology and physiology[10,11,524−531] are also important frontiers in this area. Since tunicates have a primitive backbone (a key chordate feature), these organisms are very suitable as a models for genome science which has increased the current interest in these organisms.[531] In the past few years, results have been forthcoming that put an end to the existing controversy regarding the possible existence of V(III) in biological systems and will be reviewed here.

6.1.1. Location of Vanadium in Tunicate Blood Cells

The specific form of vanadium in tunicates has been investigated since its discovery, when V(III) in cell lysates was identified in the species *Ascidia ceratodes*. The environment required to stabilize V(III) under physiological conditions would need to be either very acidic or make use of a powerful coordinating ligand. Early reports that suggested the presence of V(III) in acidic environments were met with some skepticism. The possibilities that either the acidity was an artifact of the isolation procedure or that the isolated vanadium should have been in oxidation state IV were considered and tested experimentally. These issues were compounded by subsequent studies revealing that, depending on the suborder of ascidian, as well as the cell type within each species, the form of the vanadium varied.

Although some disagreement may still exist with regard to the oxidation states of vanadium in the blood cells of ascidians, a consensus appears to have been reached: the *Aplousobranchia* suborder contains mainly V(IV) and the *Phlebobranchia* suborder contains mainly V(III). Within one organism both forms of vanadium exist depending on whether the blood cell is a lymphocyte, stem cell, leukocyte, pigment cell, or vacuolated cell. Although vanadium levels are higher than the normal levels of 10^{-9}−10^{-8} M in all these cells,[398] the vanadium is mainly stored in vacuolated cells. The vacuolated cells can be divided into nine morphologically different cell types of which three, the morula, signet ring, and compartment cells, have been the focus of many investigations. The morula cells are bright yellow mulberry-shaped cells with spherical vacuoles that exhibit orange-colored fluorescence. The signet ring cells are greenish-gray cells containing one large vacuole and the nucleus on one side of the cell. The compartment cells are green and contain several vacuoles of various sizes. Initially, it was assumed that the vanadium accumulated in the morula cells due to their intense color. However, studies using X-ray microanalysis,[532,533] cell fractionalization,[534,535] and various spectroscopic techniques[536] (reviewed in ref 7) suggest that the signet ring cells and the compartment cells are the primary storage sites. A recent study shows that vanadium also accumulates in vacuolated amoebocytes in *Phallousia mammillata* and *Ascidia sydneiensis samea*.[537]

The strong fluorescence and yellow color of the morula cells is due to a material that makes up to

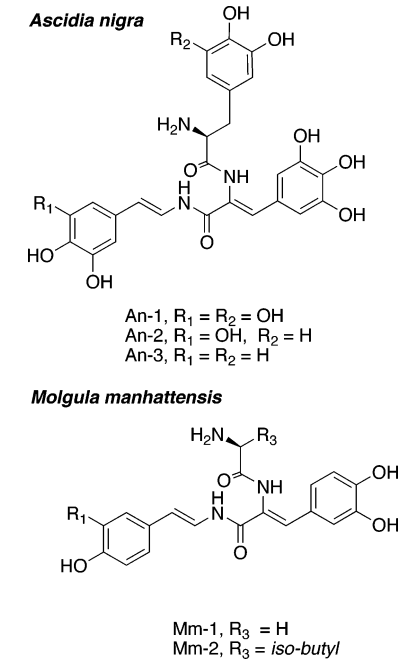


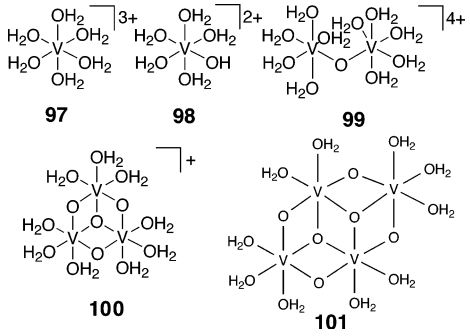
Figure 12. Selected examples of tunichrome ligands.[8,538−540]

50% of the dry weight of these cells. This material was named tunichrome, and its isolation and identification was a tour-de-force when considering the air sensitivity of both the free ligand and its colorful V-complex.[538,539] Tunichromes are polyphenolic tripeptides (Figure 12), and upon isolation yielded a naturally occurring ligand that is capable of complexing V(III) and maintain it in a low oxidation state[539,540] at physiological pH. The synthesis and possible biological roles of the various tunichromes have been recently reviewed.[8] The characterization of tunichrome initiated a series of model studies of vanadium catecholate complexes (vide infra); at the time of these early model studies it was not known that vanadium and tunichrome were located in different cells, and that little vanadium would be complexed to tunichrome.

6.1.2. Aqueous V(III) Chemistry

V(III) is the lowest oxidation state of vanadium that can be reasonably stable in aqueous solution. The aqua ion $[V(H_2O)_6]^{3+}$ can be obtained by dissolving V_2O_3 into acids or by electrolytic or chemical reduction of V(IV) or V(V) solutions. Studies of V(III) require stringent anaerobic conditions since V(III) is easily oxidized by air based on its standard reduction potential value of 0.337 V (vs NHE) in strongly acidic solutions. Experimental techniques employed for V(III) solution studies are limited to UV−Vis spectroscopy and electrochemistry. V(III) is maintained in aqueous solution only in an acidic pH range under reducing conditions or in the presence of a potent chelator.

The aqua V(III) ion is blue-green and exhibits characteristic absorption bands in the visible region at 400 nm (ϵ = 9.3) and 595 nm (ϵ = 6.0).[541] The simple $[V(H_2O)_6]^{3+}$ (**97**) and $[V(OH)(H_2O)_5]^{2+}$ (**98**) ($pK_{a1} = 2.6$) ions exist only in strongly acidic solutions pH < 1 and from 1.0 to 3.5, respectively. Upon increasing the solution pH (1.0 < pH < 3.5), V(III)



dimerizes to form $[V_2(\mu_2\text{-O})(H_2O)_{10}]^{4+}$ **(99)** ($\log K_d = 1.6$).[542] The dimer can be observed spectroscopically at 430 nm[543] with $\epsilon = 3000 \pm 50\ M^{-1}\ cm^{-1}$.[542] The dimer in acidic solution favors the bis(μ-oxo) binding mode over the bis(μ-hydroxo) binding mode.[544−546] Formation of the trimer $[V_3(OH)_8(H_2O)_{10}]^+$ (**100**) and tetramer $[V_4(OH)_{12}(H_2O)_{12}]$ (**101**) are suggested at pH > 3.5.[542] And although perhaps not yet generally accepted, the solid-state characterization of $[V_3(\mu_3\text{-O})]^{6+}$ and $[V_4(\mu_3\text{-O}_2)]^{8+}$ lend credence to the existence of trimers (**100**)[547] and tetramers (**101**)[548] in solution. An insoluble solid usually referred to as $\{V(OH)_3\}_n$ ($K_{sp} = 4 \times 10^{-35}\ M^4$)[549,550] forms at pH > 4.5 showing the need for strong chelation of any soluble V(III) that exists at neutral pH.

6.1.3. Oxidation State of Vanadium in Tunicates

The *Aplousobranchia* and *Phlebobranchia* suborders of ascidians mainly accumulate V(IV) and V(III), respectively. Most studies have focused on the latter system, in part because of the rarity of observing such a reducing metal ion in nature and also, in part, because of the experimental difficulties in identifying V(III)-containing material from biological systems. A wide range of experimental approaches have been used including spectroscopic[551−554] and magnetic[555,556] studies of isolated extracts[555,556] and whole cells[551−554,557] to convincingly demonstrate that indeed not all the observed V(III) is an experimental artifact of either the isolation or the analysis methods used.

Since V(III) is stable in acidic environments, the observation of V(III) was initially linked either to the metal ion being accumulated in acidic cells or to the metal being bound by a potent chelator. If cationic vanadium exists in cells in high concentrations, then large concentrations of counterions must also be present regardless of whether the metal was complexed to a potent ligand or not. In one study, where the V(III) in *Ascidia gemmata* blood cells contained some V(IV), analysis of the Raman SO_4^{2-} and V=O stretching peak intensities suggested a SO_4^{2-} to V^{3+} ratio of 1.47:1;[558] such a value is expected for SO_4^{2-} being a major counterion for V^{3+}. High concentrations of sulfate anion were noticed in the first studies of extracts, and have been confirmed on whole cell blood using X-ray absorption spectroscopy,[551] sulfur K-edge EXAFS[552−554] and Raman spectroscopy.[558]

Efforts at measuring and calculating the pH values of the various blood cells have been crucial for researchers in this field to elucidate the location and form of the accumulated vanadium (reviewed in ref 7). In isolated cells, these types of studies involve using markers and then monitoring the spectroscopic properties between species in cell vacuoles, cytoplasm, and extracellular fluids.[556,559−561] The morula cells are found to be neutral or slightly acidic since they contain large concentrations of acid-sensitive ligands. Most of the data for signet ring cells and compartment cells suggest that these cells are also neutral, leaving the vacuoles in these cells to be acidic and thus the location of the high concentration of vanadium. In ascidian blood cells, higher concentrations of V(III) were found to correlate with lower pH. A recent systematic and high-resolution investigation of solutions containing V(III) and sulfate at a variety of pH values; the conditions that mimic those of V(III) found in intact blood had pH values ranging from 0 to 3 depending on the sulfate content.[552,562] These studies confirm and further extend the earlier report demonstrating that V(III) does exist in whole blood cells.[551]

Recent work has focused on characterizing the nature of the V(III)−sulfate interaction in whole blood cells.[551−554,557] Although some aliphatic sulfonate[558] and low valent sulfur can be observed in intact blood cells, only traces of sulfate ester or sulfonate was found in washed blood cell membranes,[557] leading the authors to propose that sulfonate is exclusively cytosolic. Assigning coordinated sulfate to the lower C_{3v} symmetry and splitting the transition of tetrahedral SO_4^{2-} into 1s → a_1 and 1s → e transitions gives spectral fits of the data which show that chelation of sulfate to V(III) explains the natural broadening in the *A. ceratodes* blood cell sulfur K-edge XAS spectra (Figure 13).[557] Model systems have been reported showing structural precedence for monodentate and bidentate sulfate coordination as described in greater detail below.

Considering the C_{3v} symmetry of coordinated sulfate, data from intact tunicate blood cells and extracts have been modeled, and some resolution to the conflicting reports on the nature of vanadium in tunicate blood is emerging. Studies on whole cell blood of *Phallusia nigra* showed that this species has a significant fraction of the vanadium in a ligand environment provided by catechols and thus implies DOPA-like complexation.[553,562] These findings should be compared with the analysis of fresh unoxidized Henze extracts from *A. ceratodes* where 18% of the V(III) was found in a tris(catecholate)-like environment[552] consistent with the extraction causing some of the DOPA-like complexation with the V(III). Since the whole blood of these specimens was also investigated and no evidence was observed for the catechol-peptide-like (DOPA-like) complex,[552] it is clear that extraction increases the fraction of these complexes measured. Extraction is presumed to mix the contents of the morula, signet ring, and compartment cells; therefore, future work may reveal whether "leakage" of tunichrome is a beneficial event.

A recent comparison of several specimens with *A. ceratodes* from two locations shows significant differences existing even within one species.[554] Despite the anticipated biological, genetic, and environmental variability, some differences between *Ascidia* and *Phallusia* species were identified.[552,562] Importantly,

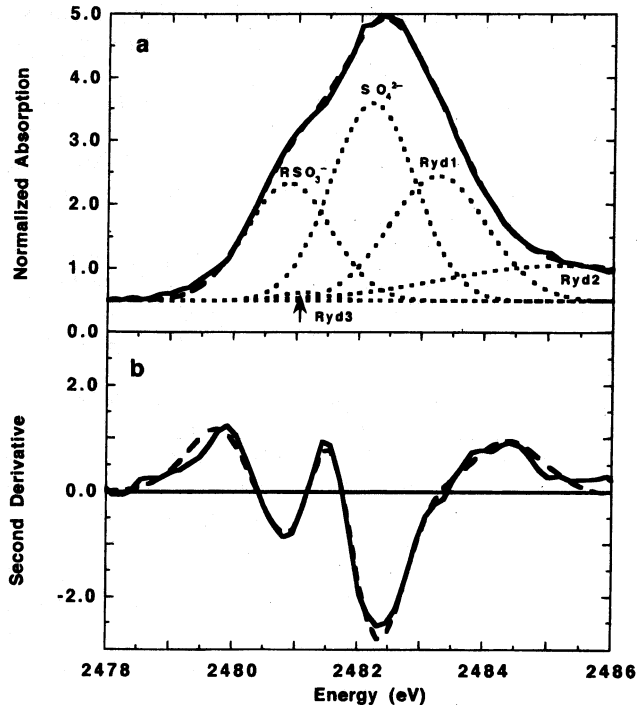


Figure 13. Sulfur K-edge XAS spectra of (a) (—) the high-valent portion of the blood cell spectrum; (– – –), the fit to the spectrum; and (- - -) the Gaussian components of the fit to the spectrum. (b) The second derivatives of (—) the blood cell sulfur K-edge XAS spectrum and (– – –) the fit to the spectrum. Reprinted from ref 557. Copyright 1995 American Chemical Society.

these studies imply that future observations of populational differences will be reported within a species and that specific blood content will vary among genera.

6.1.4. Uptake of Vanadate into Tunicates

The majority of the vanadium is taken up as V(V) from seawater (Figure 14). Since the vanadium ions inside the blood cells are in oxidation state III or IV, the aqueous vanadium has been reduced. This is not a simple feat since the V(V)/V(IV) and V(IV)/V(III) redox couples, in strongly acidic solutions, are 1.00 and 0.337 V (vs NHE), respectively. Thus, the tunicate blood cells not only support accumulation of vanadium up million-fold concentration gradients, but since no vanadium granules are observed in the

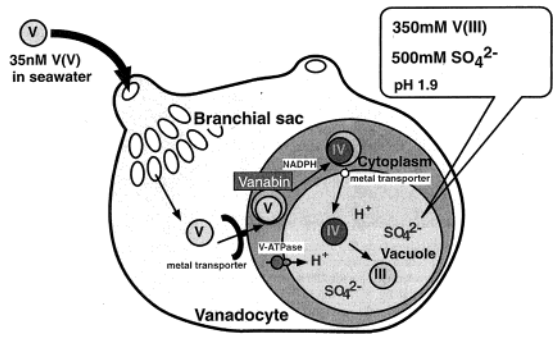


Figure 14. A model of the pathway for the reduction and accumulation of vanadium in ascidian vanadocytes. Reprinted with permission from ref 10. Copyright 2003 Elsevier.

blood cells, the V(III) will exist in the cells in a reduced form that is seemingly bioenergetically unfavorable and incompatible with physiological conditions. It has been suggested that reduction involves NADPH in the tunicate. In other biological systems, the uptake of vanadium has been linked to its reduction.[563]

Uptake studies of ^{48}V showed that vanadium is primarily transported to the tunicate's blood plasma via the branchial sacs.[564] Some additional absorption takes place in the gastrointestinal tract. Vanadate enters the tunicates through anionic transporters as evidenced by the inhibition of vanadate uptake in the presence of phosphate,[565] but recently the existence of a vanadium transporter has been suggested (Figure 14).[10] Vanadium bound to vanadium-binding proteins (vanabins) in the cytoplasm[531] may facilitate the transport and accumulation of the vanadium in the vacuoles. The fact that vanabins bind V(IV) more strongly than V(V) suggests that reduction does take place before the vanadium ion binds to the protein. ^{51}V studies show that V(V) persists in blood plasma, but blood cells trap the vanadium and facilitate the reduction to V(IV) and V(III).[59] The reduction of V(V) to V(IV) and V(III) has been of interest for some time, and model systems examine the redox properties of not only tunichrome related complexes, but other systems that may be of relevance to this reaction (vide infra).

6.1.5. Vanadium Binding Proteins: Vanabins

Three vanabins previously referred to as vanadium-associated proteins (VAPs) have been isolated.[527,530,531] The isolated vanabins include proteins with apparent molecular weights of 12.5, 15, and 16 kDa. Using a specific antibody, the proteins were shown to be localized in the cytoplasm of vanadocytes.[525,527,531,566] Two vanabins from the blood cells of *A. sydneiensis samea*, vanabin1 and vanabin2, have been cloned and characterized in great detail.[527,531] The vanabin proteins are rich in cysteine residues, but distinct from metallothioneins and other known cysteine-rich proteins.

The affinities of vanabin1 (12.5 kDa protein) and vanabin2 (15 kDa protein) for V(IV) were determined using a Bio-Gel P column and the Hummel-Dreyer method.[531] The affinities of the vanabins for V(IV) were measured using a competition experiment between the relatively weak V(IV) iminodiacetic acid complex and the proteins; dissociation constants of 2.1×10^{-5} and 2.3×10^{-5} M were obtained. Vanabin1 binds 10 vanadium atoms, whereas vanabin2 binds 20 vanadium atoms. Presumably, the differences in sequence between these two proteins are responsible for the difference in the number of protein-bound V atoms.[531] Neither Mg(II), MoO_4^{2-}, nor WO_4^{2-} affected the binding of V(IV), whereas Cu^{2+} did reduce V(IV) binding. The significance of the difference in the number of V(IV) ions bound is not clear, and it is also possible that vanabin1 also binds more V(IV) ions less tightly. At this time, EPR studies suggest (Figure 15) that the vanabins bind to the V(IV) through residues other than the cysteines.[566]

Attempts to determine the affinity of the vanabins for V(V) were not reproducible. Such reproducibility

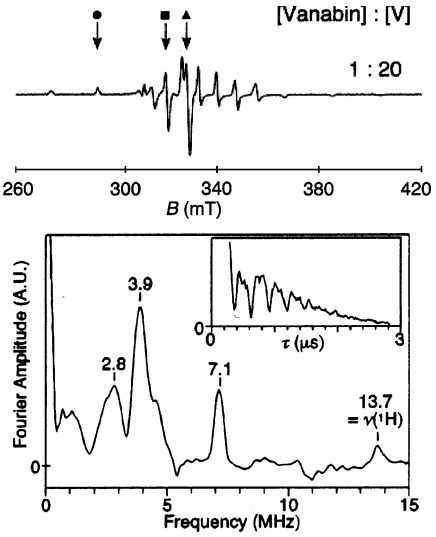


Figure 15. EPR (top) and two-pulse ESEEM spectrum (bottom) and its time-domain data spectrum (bottom inset) of VO^{2+} and vanabin2. Reprinted with permission from ref 566. Copyright 2003 American Chemical Society.

problems can be attributed to the sensitivity of the interaction between the V(V) and a cysteine-rich protein which can undergo redox chemistry resulting in protein modification and reduction of V(V) at some pH values.[567] These studies did show that the affinity of the proteins for V(V) is much less than for V(IV) and may be at the detection limit of the method employed.

The affinity of vanabins for vanadium rivals that of a nickel chaperone protein (UreE), the copper binding site of Menkes protein, and a periplasmic molybdate-binding protein (ModA). Based in part on the affinity of the vanabins for V(IV) and the location of these proteins, Michibata suggested that the roles of these vanabins are to transport the vanadium to the vacuole as illustrated in Figure 14.[10]

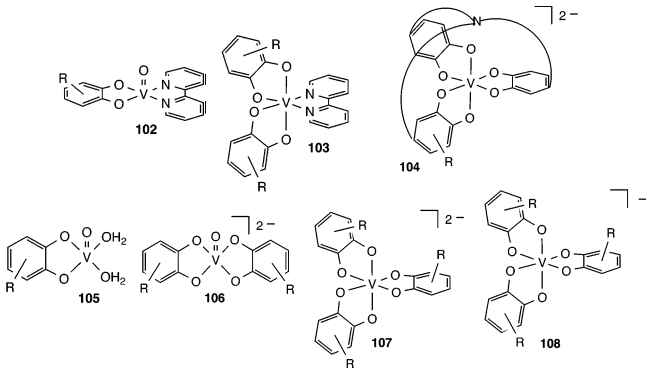
6.1.6. Model Complexes and Their Chemistry

Model studies have changed dramatically in the past decade. Initially, model studies focused on ligands that could complex V(III) and (IV), either under physiological conditions or in organic solvents. The identification of tunichrome led to a surge in activity in vanadium−catechol chemistry with a particular focus on the redox chemistry of such systems. With the determination that tunichrome and vanadium are found in different cellular compartments in *A. ceratodes*, it became clear that tunichrome is not likely to be the main natural ligand that maintains the vanadium in oxidation state III. The number of investigations into this type of chemistry has since been significantly reduced. Efforts probing the properties of this class of V-complexes now focus on those systems mimicking the potential function of ligand and V-complex. At this time, the major modeling studies in this area are those related to investigations of the properties of the vanadium−sulfate types of systems that exist in whole blood cells.

6.1.7. Catechol-Based Model Chemistry

The development and studies of model compounds flourished after the isolation and structural identification of tunichrome. Studies showed the effects of ligand modification on the structure of the V-complex and its spectroscopic and redox properties. In the tunichrome-V-complex, it is the catechol units that are coordinated to the vanadium. Model complexes for tunichrome B1 analogues demonstrated that catechol complexation is critical for high stability.[568] In the reaction of tunichrome with V(V), the reduction of V(V) to V(IV) readily took place, whereas the reduction to V(III) only happened with difficulty, if at all, under physiological conditions.[540] Details of the ligand oxidation and the metal reduction will vary with the specific catechol, although some generalizations can be made.[568,569] Several intermediates, including semiquinones and quinones, form although in some of these systems it is difficult to determine the location of the unpaired electron.[568,569]

A range of complexes (**102**−**108**) form between catechols and V(III), V(IV), and V(V); these complexes have different stoichiometries, charges, structures, nuclearities, and reactivities.[568−576] The simple V(III) catecholate and semiquinone complexes undergo a redox reaction to form dianionic 1:3 V(IV):L complexes in organic solvents and serve as an excellent model system for this class of complexes.[568,569] The V(III)-complexes, albeit more reactive, tend to be mono- or binuclear, whereas the V(IV)-complexes are often polymeric.[568,569] The generation of monomeric structures may thus require reduction to V(III)-complexes. Some of these complexes contain no oxo groups, and although nonoxo complexes are relatively rare for complexes with vanadium in oxidation state IV and V, they predominate in complexes of V(III).[577]

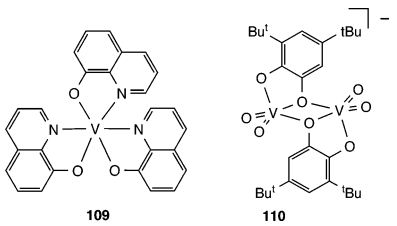


Fundamental studies of the reaction of V(V) with the simple catechols and substituted catechols are crucial for a detailed understanding of this rich redox chemistry[568,570,578−580] and have been reviewed.[246,250,540,581,582] V(V) catechol complexes have been reported in solution[578,579] and in the solid state.[461] Speciation studies have been carried out showing the formation of 1:1 (**105**), 1:2 (**106**), and 1:3 (**107**) species in various protonation states.[570−572] Although modification of the catechol does not seem to affect the formation constants of the system,[571,572] the electronic structure is affected as evidenced by the significant changes in the EPR spectrum.[583,584] Ternary complexes containing catecholate and bipyridine have many properties analogous to pure catecholate complexes.[585,586] A structurally interesting compound is the nonoxo V-complex with bipyridine

and phenanthroline as the auxiliary ligands (**103**).[461] Model compounds designed to mimic the tunichrome complex that replaced the peptide functionality failed to be as efficient in complexing vanadium.[576] However, the resulting complexes showed, as in the case of the parent tunichrome, that the catechol unit was critical for V-complex formation and dictated the properties of the parent and model systems. Recent studies with intact whole blood cells indicate that some DOPA-like ligands are coordinated to a minor fraction of the vanadium in intact blood cells, and approximately one-third of the vanadium can be attributed to these type of complexes in the *Ph. nigra* ascidians.[553] Model studies on a range of vanadium catechol and catechol-derived complexes show that when V(V) reacts with a catechol, the ligand undergoes oxidation.[246,250,540,581,582] The resulting vanadium catechol complexes contain vanadium in either oxidation states III or IV with the structures shown as in (**102**−**104**). The ligand−metal electron-transfer reaction varies depending on the solvent. Evidence for the oxidized metal/reduced quinone form is obtained in polar solvents, whereas solid-state studies support the reduced metal/oxidized ligand formulation.[170,575,577,587] Similar ligand-based redox processes were reported with other simple catechol ligands.[575,588]

An important question to consider is, how can V(III)-compounds be formed and exist under physiological conditions? Whether V(III)-complex formation is possible has been debated for some time. 3,5-Di-*tert*-butylcatechol was shown to react with $[VO(acac)_2]$ under an inert atmosphere in an organic solvent to yield a V(III) tris(semiquinone) complex.[573] 1,8-Hydroxyquinoline reduces $[VOCl_2(thf)_2]$ in the presence of Et_3N to yield $[V^{III}(quin)_3]$ (**109**).[574] Disproportionation reactions (and enzymatically catalyzed reactions) are known to facilitate processes that may otherwise be difficult; such disproportionation reactions have been observed in vanadium−salen complexes (eqs 10 and 11). This reaction needs to be considered in detail since there has been some concern as to whether physiologically relevant reducing agents such as glutathione, NADH, and NADPH are competent to reduce V(IV)-complexes to V(III).[7] Initial results obtained by EPR spectroscopy suggested that the *Molgula manhattensis* tunichrome reduces, in vitro, V(V) and V(IV) to V(III).[540] However, further reexamination by means of a calorimetric V(III) assay revealed that both *M. manhattenis* and *Ascidia nigra* tunichromes failed to produce detectable amounts of V(III) in either acidic (pH 2) or neutral (pH 7) media.[582] The possibility that *A. nigra* tunichrome assayed with V(V) at pH 7 generated low levels of V(III) was, however, not ruled out.[582] Interestingly, a cysteine methyl ester was recently shown to be able to reduce V(IV) to V(III) in the presence of aminopolycarboxylates.[589] The search for a natural reductant of V(IV) to yield V(III) continues. The difficulties experienced in designing such systems provides fuel to the controversy regarding the reductive power of tunichrome-type ligands[540,582] and other ligand systems.[589]

New complexes of vanadium with catechols and similar units continue to be reported,[306,478,571,572,590]



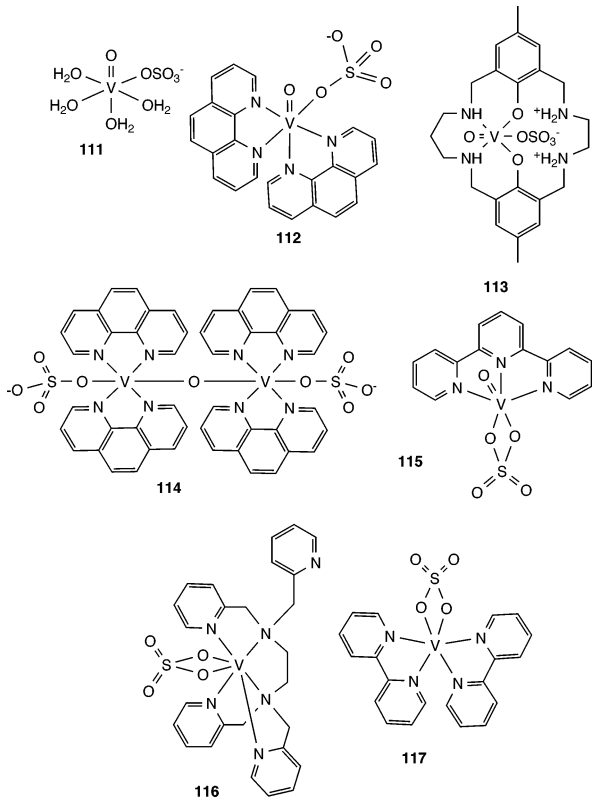
$$2V^{IV}O(salen) + 4H^+ \rightarrow V^{V}O(salen)^+ + \text{“}V^{III}(H_2salen)\text{”} + H_2O \quad (10)$$

$$V^{V}O(salen)^+ + \text{“}V^{III}(H_2salen)\text{”} + 4Cl^- \rightarrow V^{IV}O(salen) + V^{IV}Cl_2(salen) + 2HCl \quad (11)$$

including a dinuclear V(V) catecholate complex $[Et_3NH]_2[VO_2(3,5\text{-dtbc})]_2$ (**110**).[574,575] Because of their rich properties, these systems have versatile reactivity patterns, some of which will be described as model systems for the nitrogenase reaction.[591−594]

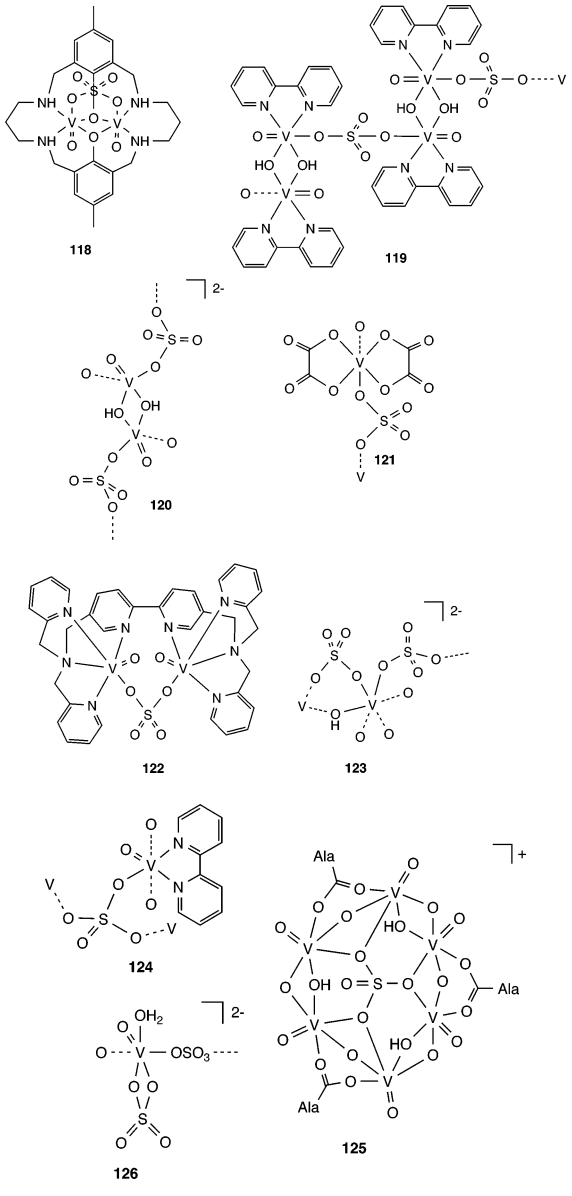
6.1.8. Vanadium Sulfate Complexes

The structural and functional properties of vanadium-sulfate model compounds have been of increasing interest to better understand the nature of the vanadium in the blood cells of these animals. The recent interpretations of the XAS spectra (Figure 13) suggest that the presence of the sulfate is responsible for the line broadening observed,[557] and begged the need for comparison with spectra from complexes also characterized in the solid state by X-ray crystallography.



Representative examples of structurally characterized vanadium−sulfate compounds are shown (**111**−**117**). Most of the structural studies of vanadium−

sulfate interactions have focused on V(IV) presumably due to the oxygen sensitivity of V(III) and the lower affinity of sulfate for V(V). The structural information summarized here is mainly based on crystallographic information although a symmetry analysis of IR bands suggests that a distinction between bridging and chelating sulfate groups can be made in many cases.[595] Sulfate can interact in a variety of ways with vanadium. Structural information is available on monodentate (**111**−**114**), bidentate (**115**−**117**), and several bridging coordination modes to two (**118**−**123**), three (**124**), and even six (**125**) vanadium



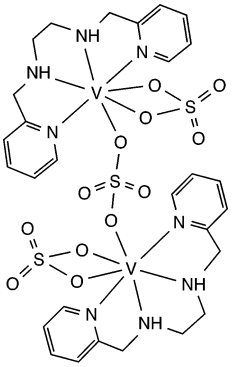
centers; even a mixed bidentate/bridging coordination mode (**126**, **127**) has been observed. There are additional examples of purely inorganic crystal structures containing vanadium−sulfate interactions. Most of these compounds are obtained from hydrothermal syntheses, and their structures contain sulfate-bound vanadium in oxidation states V, IV, III, and II with bonding patterns similar to those summarized above. Representative examples are shown of these purely inorganic structures model industrial catalysts in sulfuric acid productions (**111**, **120**, **125**, and **126**). Many of these interactions will not remain intact in solution.

An aqueous vanadyl complex with sulfate anion, $[VO(H_2O)_4(SO_4)]$, was prepared via hydrothermal synthesis and contained layers of complex, sulfate, and piperazinium ions (**111**).[596] In many sulfate-bound V(IV) structures, pyridyl-type ligands are effective stabilizing ligands,[597−601] although other ligands can be used. A monomeric vanadyl complex with bidentate sulfate, $[VO(terpy)SO_4]$ was structurally characterized (**115**).[597] A density functional theory (DFT) analysis indicated that in the gas phase $[VO(terpy)]^{2+}$ unit favors coordination with SO_4^{2-} over coordination with two water molecules by ∼350 kcal/mol.[597] A series of neutral phenanthroline oxovanadium(IV) complexes with monodentate sulfate ligands have been structurally characterized (**112**); the integrity of these complexes under physiological conditions was not discussed even though anti-leukemic activity was reported.[598]

Bi- and polydentate sulfate-containing ligands have been used as stabilizing agents in several studies to generate binuclear complexes. Vanadyl−sulfate complexes (**113**),[602,603] sulfate-bridged divanadyl complexes (**118**),[602,604] and vanadyl−heterometallic sulfate complexes[602,604] are among this class of complexes that have been isolated and characterized. A tetraoxo-tetravanadium(IV) cluster with a tri(hydroxy)-alkyl ligand and a bridging sulfate[605] and a related (μ_6-SO_4) polyoxometalate cluster (**125**) were synthesized from an aqueous solution of V_2O_5 and β-alanine.[606] A range of polymeric chains expressed as $\{[V^{IV}O(SO_4)(bipy)]\}_\infty$, (**124**),[599] $\{[V^{IV}_2O_2(\mu\text{-}OH)_2(bipy)_2]\text{-}\mu\text{-}SO_4\text{-}[V^{IV}_2O_2(\mu\text{-}OH)_2(bipy)_2]\}_\infty$, (**119**),[600] $\{[(V^{IV}O)_2(OH)_2(SO_4)_2]^{2-}\}_\infty$, (**120**),[607] and $\{[V^{IV}O(H_2O)(SO_4)_2]^{2-}\}_\infty$, (**126**)[607] were all prepared by hydrothermal syntheses and have been structurally characterized. In $\{[V^{IV}O(H_2O)(SO_4)_2]^{2-}\}_\infty$, (**126**), the vanadyl group is bound by a bidentate sulfate group and a bridging sulfate group;[607] in $\{[V^{IV}O(SO_4)(bipy)]\}_\infty$, (**124**), the sulfate acts as a tridentate bridge.[599]

There are fewer structures containing V(III) in line with the greater air sensitivity of this oxidation state and the fact that sulfate fails to stabilize the reduced vanadium sufficiently. A coordination polymer of sulfate-bridged dioxalatovanadate(III), *trans*-$K_3[V(ox)_2(SO_4)]_n$, (**121**) was structurally characterized in contrast to a monomeric complex *trans*-$K_5[V(ox)_2(SO_4)_2]$ that was only characterized by IR and Raman spectroscopy.[608] Using the *N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)ethylenediamine ligand, (tpen), a hexacoordinate V(III)-complex with a bidentate sulfate group was isolated (**116**) and was found to be stable in aqueous solution.[601] If *N*,*N*′-bis(2-pyridylmethyl)-ethylenediamine, (bispicen), was used instead, a dimeric, heptacoordinate V(III)-complex with a bridging sulfate was isolated (**127**).[601] The latter of the two complexes is hydrolytically unstable and forms $[V_2Cl_2(\mu\text{-}O)(bispicen)_2]Cl_2$ in aqueous solutions.[601] A potentially octadentate polypyridyl ligand binds two vanadyl cations, which are further linked by a bridging sulfate anion (**122**).[609] An oxo-bridged V(III) dimer, $\{[V(phen)_2(SO_4)]_2O\}$, prepared from a hydrothermal reaction of V(V), contains monodentate

sulfate coordinated to the vanadium centers (**114**).[610] A polymeric material with repeating $[V^{III}(OH)(SO_4)_2]^{2-}$ units has also been recently obtained (**123**).[607] Other inorganic V(III) sulfate complexes have been structurally and spectroscopically characterized,[611−615] including the anhydrous $V_2(SO_4)_3$ salt.[616]



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While V(II)-compounds are not likely to exist in biology, they are discussed briefly for completeness and for comparison with V(III)-compounds. One organic V(II) sulfate complex, $[V(bipy)_2(SO_4)]$, has been crystallographically characterized and shows a bidentate sulfate ligand (**117**).[617] The corresponding $[V(py)_4SO_4]$ compound was also synthesized. However, only a small number of inorganic V(II) sulfate compounds have been structurally characterized.[618,619]

V(IV) rapidly forms a 1:1 complex with sulfate ($K_f = 300\ M^{-1}$).[620] V(III) in contrast to V(IV) has a modest affinity for sulfate ions in aqueous solutions. V(III) speciation studies have been carried out using spectrophotometry, redox measurements, and potentiometry.[542] The 1:1 ($[V(SO_4)(H_2O)_5]^+$, $K \approx 30\ M^{-1}$) and 1:2 species ($[V(SO_4)_2(H_2O)_4]^-$, $K \approx 2-3\ M^{-2}$) have reasonable stability at acidic pH, although at mildly acidic pH their hydrolysis products ($[V(OH)(SO_4)(H_2O)_4]$, $pK_{a1} = 3.2$ and $[V(OH)_2(SO_4)(H_2O)_4]^-$, $pK_{a2} = 4.6$) form.

Structural features of V-sulfate complexes can be summarized as follows. Complexes in which the sulfate is monodentate contain a V=O group. When sulfate coordinates in a bidentate manner, the vanadium can contain a V=O group or be a nonoxo vanadium depending on the ternary ligand bound to the vanadium. In cases in which the bidentate sulfate group bridges two vanadium atoms, the vanadium contains the V=O unit. Such dinuclear structures can be discrete molecules or part of an extended network that may support extensive hydrogen bonding. Triply bridging sulfate is more rare and includes structures in which the vanadium atoms are bound to more than one type of sulfate. These structures are relatively inorganic in nature and are consistent with an inorganic coordination environment inside the tunicate vacuoles. Any organic ligand to vanadium that might be present in the vacuoles should be able to withstand acidic environments, have low pK_a values, and be able to effectively compete with sulfate complexation; pyridyl-containing biomolecules are potential ligands.[597]

6.2. Fan Worm *Pseudopotamilla Occelata*

The fan worm *Ps. occelata* of the *Polychaeta* class was reported to contain high levels of vanadium in 1993.[519] The vanadium concentration is highest in the bipinnate radiole, the epidermis covering the radioles. Little vanadium was found in the muscular, connecting, and supporting tissues or in the blood plasma, whereas vanadium accumulation on the vacuolar membranes and in intravacuolar and cytoplasmic electron-dense inclusions were observed. Most of the vanadium is believed to be in oxidation state III and a high level of sulfate is found in the vanadium-containing cells.[520,521] Although the vanadocytes are quite different between ascidians and this fan worm, it is presumably significant that the storage locale and form seem to be the same in these animals.[520,521] The role of vanadium in these animals is not known. Proposals include roles in the regulation of oxidation−reduction reactions at the surface of the radiole, in the absorption of O_2, and in detoxification mechanisms. The possibility that the action of V-compounds occurs prior to their accumulation in the vanadocytes was suggested.[520]

7. Vanadium Nitrogenase

7.1. Nitrogenases

The bacterial enzyme nitrogenase (Nase) is capable of catalyzing the reduction of atmospheric N_2 to NH_3 and is responsible for cycling about 10^8 tons of N per year from the atmosphere to the soil.[27] Nase is a metalloprotein containing Mo and Fe, V, and Fe or Fe only as the metal cofactors. The enzymatic N_2 fixation occurs at ambient temperature and 0.8 atm N_2 pressure; modeling this process remains one of the great challenges for bioinorganic chemists. The fascination with this protein can be explained, in part, because of the several protein and inorganic components, and in part because of the complex chemical reaction that is catalyzed by this enzyme. The overall reaction of the common molybdenum-containing Nase is currently believed to be as shown in eq 12. Some variation in the stoichiometry of e^-, H^+, and MgATP's and the overall products of the reaction exists depending on the specific conditions under which the reaction occurs and which Nase is used. A greater amount of H_2 is generated for each NH_3 produced and more ATP is consumed during the reduction of N_2 by the vanadium nitrogenase (V−Nase) (eq 13) as compared to the molybdenum nitrogenase (Mo−Nase) (eq 12).[27] Whether the exact number of MgATP required for the V−Nase reaction is 40[27,30] or 24[28−30] can presumably be traced to the difficulties in the preparation and purification of the enzyme. The enzymes carry out their function under anaerobic conditions and have developed elaborate protection mechanisms to exclude oxygen from the active site and the redox active cofactors.

$$N_2 + 8e^- + 8H^+ + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i \quad (12)$$

$$N_2 + 12e^- + 14H^+ + 40\ (\text{or } 24)\ MgATP \rightarrow 2NH_3 + 3H_2 + 40\ (\text{or } 24)\ MgADP + 40\ (\text{or } 24)\ P_i \quad (13)$$

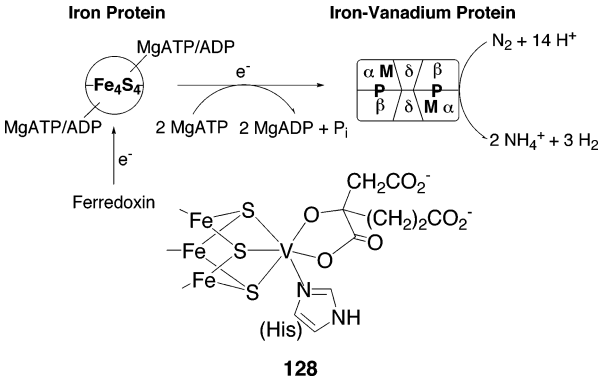

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Figure 16. Schematic representation of V−Nase and the possible structure of vanadium in FeVco. Redrawn from ref 28. Copyright 2000 Elsevier.

Three classes of Nases exist and all of them are comprised of Fe−S-cluster-containing subunits, but differences exist with regard to the heterometal-containing protein cofactor. The V−Nase is comprised of an Fe protein and a V−Fe protein, and this enzyme is the focus of this review. The other Nases, which have been described in detail elsewhere,[7,27,51,621] will only be discussed when illuminating the function or properties of the V−Nase. Studies on V−Nase have been plagued by the recognition that apparent homogeneous mixtures of protein preparations have in fact been mixtures, and have hindered mechanistic and structural studies.[27]

The largest class of Nases is the *nif*-encoded Nase, which contains molybdenum and iron in the heterometal cofactor. This type of Nase is expressed under normal conditions, and is referred to as Mo−Nase. The second largest class of Nases is the *vnf*-encoded Nase. This Nase contains vanadium and iron in the heterometal cofactor, and is referred to as V−Nase. Although V−Nase is widely distributed, it is only expressed when Mo is a limiting nutrient. The third Nase is the *anf*-encoded Nase that only contains iron in its active site and in the Fe−S-cluster cofactors. This Nase is the most difficult one to access and is only expressed under molybdenum and vanadium starvation conditions in some microorganisms. This Nase is referred to as the Fe−Nase. Although VNase is a distinct enzyme, its heterometal-based active site is structurally analogous to that of Mo−Nase.

7.2. Biochemistry of Nitrogenase

The Nases contain two major protein components, and each of them is comprised of multiple subunits and/or metal clusters (Figure 16). One protein complex is an iron-containing protein, and another is the heterometallic protein containing molybdenum (MoFe), vanadium (VFe), or iron (FeFe). Specifically, in the V−Nases, the homodimeric Fe protein has a MW of 64 kDa and the VFe protein has a MW of 240 kDa.[622] The heterometal-containing subunit has two different types of inorganic clusters, a P cluster (Fe_8S_7) and an MFe-cofactor (Fe_7S_9M, M = Mo, V, Fe). The V form is exclusively observed in the V−Nase and the Fe form is exclusively observed in the Fe−Nase.

The iron protein component contains two α subunits, which are bridged by a [Fe_4S_4] cluster and incorporates two binding sites for MgATP. A schematic illustration of functional aspects of the complex is shown in Figure 16. Investigations into the enzymology of this process have recently been reexamined using ATP-analogues after it was recognized that this protein contains the peptide fold common for nucleotide binding proteins.[623,624]

The FeV component has a $\alpha_2\beta_2\delta_2$ subunit structure, and contains two P clusters at the α and β subunit interface. The P cluster is believed to transfer electrons to the FeM-cluster in all three Nases. The M cluster in the V−Nase is commonly referred to as FeVco and is the proposed binding site for N_2. Two FeVco clusters are located in the α subunits (Figure 16). The FeVco cluster can be described as the catalytic cofactor since it is responsible for the conversion of N_2 to NH_3 (vide infra). The exact structure of the VFe protein is still not known, but all studies so far strongly suggest that it is analogous to the Mo−Nase.[625] A second V−Nase has been found in *Az. vinelandii*, which lacks the α subunit and half of the P cluster, but is active nonetheless.[626,627] Thus far, all the clusters have only been assembled by biosynthesis.

In the absence of a crystal structure of a VFe protein, the detail of the environment of the V-binding site has been provided from EXAFS studies of proteins isolated from *Azotobacter chroococcum* ($Ac1^v$)[628] and *Az. vinelandii* ($Av1^v$).[629] The V-atom was in an environment similar to that observed for the V-atom in the model compound $[Me_4N][VFe_3S_4Cl_3(dmf)_3]$ (**129**).[630,631] XANES and EXAFS studies of thionine-oxidized and dithionite-reduced forms of the *Az. chroococcum* Nase suggest a vanadium oxidation state between +II and +IV.[628,632] EPR studies in reduced *Az. vinelandii* V−Nase give $g = 5.5$, which corresponds to an overall $S = 3/2$ spin for the cluster in the protein.[51,633]

Temperature has been shown to play a role not only with regard to the activity of the Nases, but also in the expression of the enzymes themselves. Upon increasing the temperature from 30 to 40 °C, the amount of N_2H_4 produced by the *Az. chroococcum* V−Nase increased by a factor of 15, whereas the amount of NH_3 produced remained constant.[634] Increasing the temperature to 50 °C resulted in a further 3-fold increase in N_2H_4 production and a 7-fold decrease in NH_3 production.[635] These reversible effects were interpreted as temperature-induced conformational changes, which at high temperatures, may allow FeVco to reduce N_2 to N_2H_4, but not to NH_3.[51,634] In the same system, V−Nase was shown to have 10 times the activity of the Mo−Nase when the temperature was lowered to 5 °C.[636] In the *Az. vinelandii* enzyme system, at 30 °C, Mo was found to repress both the *vnf* and *anf* operons, less so at 20 °C, and not at all at 14 °C.[637] Furthermore, vanadium was shown to repress the *anf* operon at 30 °C, but not at 20 °C; this suggests that *Az. vinelandii* can produce all three Nases between 14 and 20 °C, depending on the availability of Mo and V. These results suggest that Nases found in cooler climates may favor the presence of V-Nases.

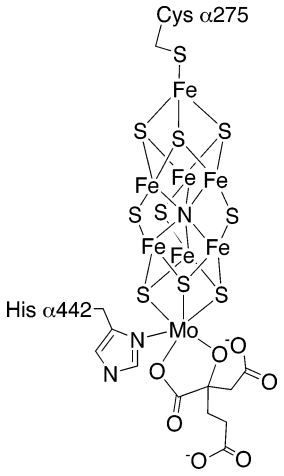


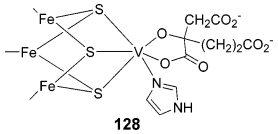
Figure 17. Structure of the FeMo-cofactor of dithionite-reduced *A. vinelandii* MoFe-protein. Redrawn from ref 641. Copyright 2002 American Association for the Advancement of Science.

7.3. Clusters in Nitrogenase and Model Systems: Structure and Reactivity

The P and FeMoco clusters have been structurally characterized for the Mo−Nase;[53,638−640] however, recently the MoFe-protein was investigated at 1.16 Å resolution and an interstitial atom located within the Fe_6 core (Figure 17) was discovered.[641] This new structure has provided information on which all previous data needs reconsideration, given the role and effect such a trapped element would have. Calculations have been carried out and provide support for the expectation that a nitrogen atom is the best candidate.[52,642] ESEEM spectroscopy showed that the trapped element did not exchange with labeled N_2 during catalysis,[643] suggesting that the cluster remains intact and does not exchange with N_2 during catalysis. Although less likely, the possibility that exchange did not take place because the interstitial element is something other than nitrogen has not yet been ruled out. Discovery of a trapped nitrogen atom is directly relevant to the heterometal core in FeVco because of its structural analogy to the heterometal core of FeMoco.[27,51,625] Model calculations investigating the structure and reactivity of the active site Nase cofactor cluster, MFe_7S_9, were carried out. The studies indicated that the size of the cofactor cluster is dependent on the nature of the heterometal and increases in the order FeMoco < FeVco < FeFeco.[644]

The P cluster in Mo-nitrogenease has a stoichiometry of Fe_8S_7 and is closely related to the iron−molybdenum (FeMoco) and iron−vanadium (FeVco) clusters containing an MFe_7S_9 structure with a heterometal and two cysteine residues added to the P cluster geometry. The P cluster can also be represented in combination with two nearby cysteine residues from the protein to become a cluster with an Fe_8S_9 structure. Recently, synthetic clusters of this system have been characterized in detail based on synthetic methodology developed for the preparation of the MFe_4S_6 cluster.[645−649] The P cluster can be obtained in a reduced and oxidized form: the P^N cluster being the two-electron reduced form of the P^{OX} cluster.[646−649]

The active site in the Mo−Nase cofactor is a $MoFe_7S_9N$ cluster (Figure 17). A corresponding partial structure has been proposed for the FeVco (**128** in Figure 16) and is supported by vanadium EXAFS and other investigations.[625,628,632] Such a proposal must now consider the presence of an interstitial nitrogen atom.[641] These studies and interpretations implicate coordination by sulfides, homocitrate hydroxylate and carboxylate groups, and a histidyl imidazole group (**128** in Figure 16). The heterometal cubane-type clusters with $[MFe_3S_4]^{z-}$ cores (for example, **129**) have been useful precursors in assembling synthetic analogues of the FeMoco and FeVco clusters of the Nase cofactors.[650] For example, the reduced cores with the framework $[VFe_3S_4]^{2+}$ are topological analogues of the reduced clusters of Nase and are effective in demonstrating the redox properties of these types of clusters and the analogous Mo-containing clusters.[646−649]



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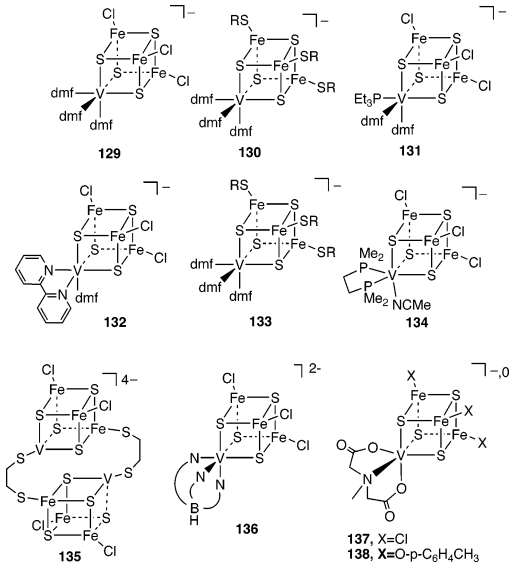
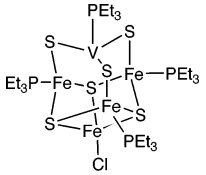
The first vanadium-containing model system reported, the VFe_3S_4-cluster $[(dmf)_3VFe_3S_4Cl_3]^-$, (**129**),[630,651] illustrated the stability of the $[VFe_3S_4]^{2+}$ unit, since it formed in cluster self-assembly and remained intact in the presence of excess ligand. A series of clusters, (**130**−**136**), related to this cluster were subsequently reported and the structures and reactivity of these species investigated in detail.[631,651−653] Important new clusters that have been reported include two vanadium clusters with the VS_3O_2N coordination unit, $[(Meida)VFe_3S_4Cl_3]^{3-}$ (**137**) and $[(Meida)VFe_3S_4(O\text{-}p\text{-}C_6H_4CH_3)_3]^{3-}$ (**138**),[650] (Meida = *N*-methyliminodiacetic acid) as well as the first synthetic cluster with the exact core composition as FeMoco, $\{[(Meida)MoFe_3S_4Cl_2)](\mu_2\text{-}S)(Fe_4S_4Cl_3)\}^{4-}$.[650]



When V(III) is incorporated into an iron−sulfur cluster as $[VFe_3S_4]^{2+}$ a different charge distribution results $[V^{3+}Fe^{3+}Fe^{2+}{}_2S_4]^{2+}(Fe^{2.33+})$.[649] Examples of such clusters, $[(dmf)_3VFe_3S_4Cl_3]^-$ (**130**), $[(dmf)_2(PEt_3)VFe_3S_4Cl_3]^-$ (**131**), and $[(dmf)(bipy)VFe_3S_4Cl_3]^-$ (**132**), are capable of catalyzing the reduction of hydrazine by cobaltocene/lutidine hydrochloride.[651,653−655] Reduction of hydrazine to NH_3 is commonly used to document the reactivity of these clusters and for the cuboidal clusters (**130**−**135**); the coordination of the hydrazine to the V-atom appears to be critical to its reduction.[630,651,653,655] In contrast to the complexes (**129**−**135**) described above, $[(HBpz)_3VFe_3S_4Cl_3]^{2-}$ (**136**)[653] does not catalyze hydrazine reduction. The inactivity of this complex is consistent with the interpretation that the catalytic reduction rate of hydrazine of these complexes in general depends on the terminal ligands coordinated to vanadium. That is, fewer labile solvent molecules coordinated to the V-atom lead to lower rates of hydrazine reduction.

A new class of clusters containing the MFe_4S_6-unit was reported and represents a new topological system slightly closer to the FeVco cluster.[645] The $[VFe_4S_6(PEt_3)_4Cl]$ complex (**139**) was compared in detail to the corresponding Mo-system, and due to the large cavity size in this cluster, greater differences were observed between the different heteroatom-containing systems. The openness of this structure is in dramatic contrast to the recently discovered interstitial nitrogen atom in FeMoco, and may explain the reactivity of (**139**), which is distinct from that observed with the VFe_4S_6-unit.



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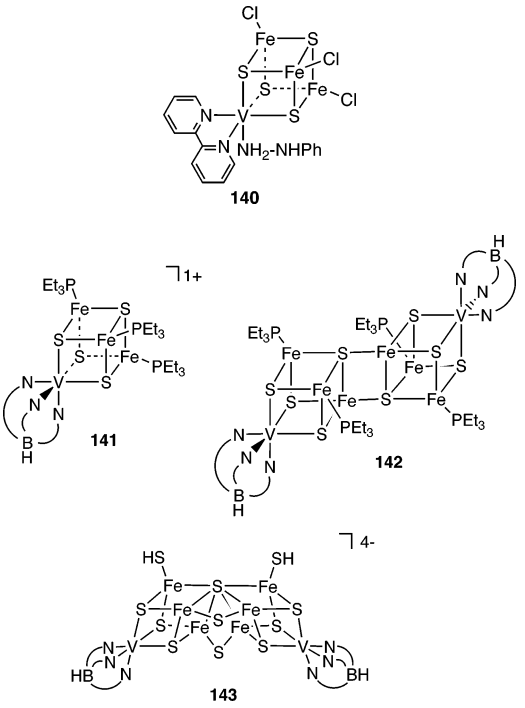
These clusters also catalyze the reduction of phenylhydrazine to NH_3 and aniline. Attempts to characterize intermediates by the isolation and characterization of complexes with hydrazine have led to the report of the hydrazine vanadium single cubane complex $(Me_4N)[(PhHNNH_2)(bpy)VFe_3S_4Cl_4]$ (**140**).[655] A related cluster (**141**) reacts to form (**142**) and subsequently (**143**).[648,649] The S-bridged structure of $[(Bpz)_2V_2Fe_6S_9(SH)_2]^{4-}$ (**143**) has crystallographically imposed C_2 symmetry. The core exhibits a bridging pattern $[V_2Fe_6(\mu_2\text{-}S)_2(\mu_3\text{-}S)_6(\mu_6\text{-}S)]$, which reduces to two cuboidal $VFe_3(\mu_3\text{-}S)_3$ fragments that share a common bridging atom (the μ_6-S atom) and are externally bridged by two μ_2-S atoms. Cyclic voltammetry experiments on the $[VFe_3S_4]^{2+}$ cluster, $[(HBpz)_3VFe_3S_4(LS_3)]^{2-}$ (**136**), demonstrated the stability of this system by reversible conversion among the 1+, 2+, and 3+ oxidation states of the clusters.

Double-cubane clusters such as $[V_2Fe_6S_8Cl_4(C_2H_4S_2)_2]^{4-}$ (**135**)[652] have been reported and their reactivity investigated. When this cluster reacts with $HSCH_2CH_2SH$ in dmso, a single cubane-cluster was formed (**133**). Single and double cubane clusters in multiple oxidation states were isolated from $[(HBpz)_3VFe_3S_4(LS_3)]^{2-}$. The $[VFe_3S_4]^{3+}$ unit with the formal charge distribution of $[V^{3+}Fe^{3+}{}_2Fe^{2+}S_4]^{3+}$, $(Fe^{2.67+})$,[649] was obtained from the double-cubane cluster $[V_2Fe_6S_8(SEt)_9]^{3-}$ [656] and the single cubane cluster $[VFe_3S_4(Et_2dtc)_4]^-$.[283] Recently, new edge-bridged double cubane clusters $[(HBpz_3)_2V_2Fe_6S_8(PEt_3)_4]$ (**142**), and $(Et_4N)_4[(HBpz_3)_2V_2Fe_6S_8Cl_4]$,[648,649] were characterized. This study allows the transformations (**136**) → (**141**) → (**142**) → (**143**) to be monitored and document the feasibility of reversible redox changes in the clusters.[648,649] These observations support the recent findings that the P cluster may change during enzyme catalysis.[648,649]

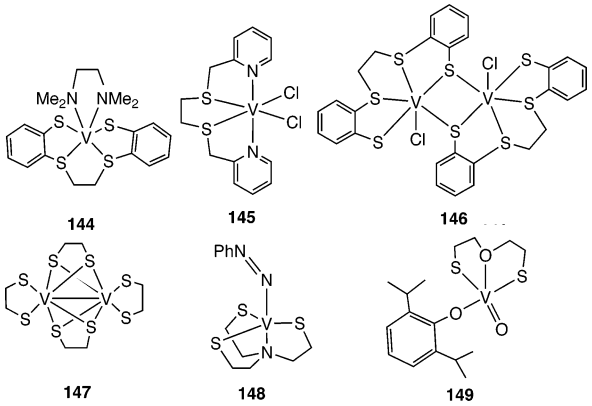


140 **141** **142** **143**

7.4. Structural Model Complexes of the VFe Cofactor Binding Site

In the V−Nase, the coordination sphere of the FeVco cofactor contains three sulfur atoms from Fe−S cluster, one N-atom from His-421, and two O-atoms from (*R*)-homocitrate (**128**). There will also likely be three Fe-atoms near the V-center with V−Fe distance of 2.76 Å.[27,28] Complexes have been reported that model part of this structure. The ligands to the V-atom in these complexes are sulfur, nitrogen, or oxygen atoms (**144**−**147**). The valence of the V-atom ranges from +III to +V and thus includes model complexes that have limited air sensitivity. Complexes with a variety of bi- and tetradentate S-donor functionalities have been prepared as model complexes for Nase.[657−660] These complexes have some structural similarities to the binding site of Nase. However, in contrast to the isolated cofactor, they fail to convert N_2 to NH_3. Selected structures of this type are shown (**144**−**147**). Additional S-coordinated vanadium coordination compounds from V(II) to V(V) have been described in previous reviews.[9,16,28] V(III) and V(V) complexes of tris(2-thiolatoethyl)amine $N(CH_2CH_2S)_3^{3-}$ (NS_3) (**148**) and bis(2-thiolatoethyl)-ether $O(CH_2CH_2S)_2^{2-}$ (**149**) are representative of

complexes coordinated to a coligand and may be related to the nitrogen fixation process.[661,662] These coligands can be hydrazine, hydrazide, imide, amine, organic cyanide, and isocyanide. Since none of these can convert N_2 to NH_3, these complexes serve only as structural models.[16,28]



7.5. Homocitrate

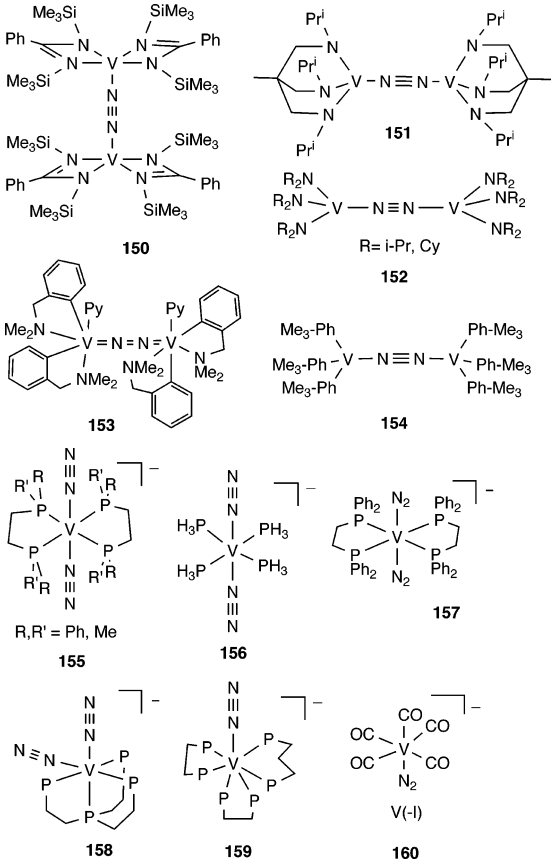
Homocitrate is a noncovalent cofactor and a structural component of FeMoco. It is presumably also a component of FeVco and the iron−iron (FeFeco) clusters, because nifV is required for full functionality of all three Nases.[663] A ^{49}V labeled study showed that homocitrate is required for the V-precursor transfer of FeVco from VnfX to *nif*-apodinitrogenase.[664] The dinuclear V(V) species $[K_2(H_2O)_5][(VO_2)_2(R,S\text{-homocitrate})_2]\cdot H_2O$ represents the first synthetic and structural characterization of a transition metal homocitrate complex and may in fact be an early precursor in the biosynthesis of FeVco.[490]

For comparison, several V(V)-citrate complexes have been reported.[480,483,490] The dinuclear V(V)-citrate complex, $K_2[V(O)_2(C_6H_6O_7)]_2\cdot 4H_2O$, demonstrated the coordination mode found in the Nase cofactor.[483] See the siderophore section of this review for additional details on V-citrate complexes.

7.6. Activation of Nitrogen

Given the inertness of N_2, the formation of molecular nitrogen coordination compounds is a key step in nitrogen fixation. The ability of V(II) to reduce N_2 to NH_3 and/or hydrazine was shown more than 30 years ago.[665−667] Shilov demonstrated that N_2 is reduced in alkaline solution in the presence of mixed V(II) and Mg(II) gels[667] and in alkaline V(II)-pyrocatechol solution.[666] Although many transition metal nitrogen complexes have been synthesized, (**150**−**159**), most of these such as $[V(CO)_5N_2]^-$ (**160**) are only stable in solution below −53 °C.[668] Bioinorganic chemists are far from their goal of generating catalytically competent complexes for nitrogen fixation that are stable at ambient temperature.

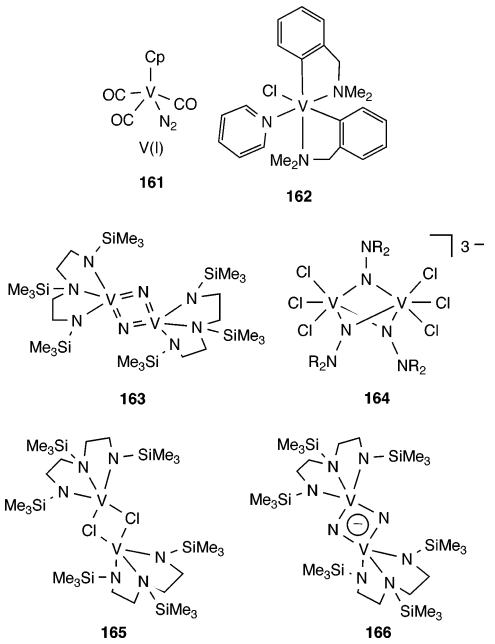
The activation of N_2, by forming bridging (**150**−**154**) or end-on (**155**−**161**) N_2 adducts in dinuclear and mononuclear metal complexes, has been reported and characterized.[668,669] This type of reaction works best with octahedral complexes of V(II) and V(III),



which tend to have high spin states of the dinuclear metal complexes, thus favoring interactions between the nitrogen $1\pi_g$ and the metal d orbitals.[670,671] The activation of N_2 can range from poor to strong depending on the charge of the complex, the presence of alkali atoms and the spin state of the complex.[670,671] Ab initio calculations show dinuclear complexes with the V−N−N−V motif exhibiting considerable V−N interactions, with V(II)-complexes being more stable than V(III)-complexes.[670,671] The preparation and characterization of a bridging complex, with the unit V(III)−N_2−V(III) (**151**), has however, been reported.[669] In mononuclear V(II)- and V(III)-complexes, the V−N interaction is less stable due to the weak nitrogen antibonding $1\pi_g$ interactions with the appropriate metal d orbital. Not surprisingly, no mononuclear V(III)−N_2 complexes have been prepared; however, mononuclear V(−I)-complexes exist (**155**−**161**).

The series of V(−I)-complexes expand the known systems to include a class of complexes with monodentate (**156**, **160**, **161**), bidentate (**155**, **157**, **159**), and tetradentate (**158**) phosphine ligands.[672] In complexes with more than one N_2 functionality, the N_2 unit can be cis or trans. The cis isomers generally are the kinetic products, while the trans isomers are typically the thermodynamic products. Inspection of the spectroscopic data indicates an ion-pair interaction of the type V−N═N···M^+.[672] Results from computational studies on the model complex $[V(PH_3)_4(N_2)_2]^-$ (**156**) suggest that the anionic vanadium center favors metal-to-ligand π-back-bonding while hampering ligand-to-metal σ donation.[673] This is consistent with observations of the reductive protonation of these complexes to form NH_4^+ [672,674,675] and

the ability of CO and isocyanides to easily displace coordinated N_2.[672] Amides and amidinate ligands ($RNC(R')NR^-$) have been employed as versatile precursors for dinuclear compounds with metal−metal bonds such as the N_2 bridged dinuclear V-complexes shown in **150**−**154**.[676] The diazenido(2-)-bridged complex in **150** has V−N and N−N bond lengths of 1.76 and 1.24 Å, respectively, but these dinuclear complexes cleave upon the addition of thf. Coordination of other donor ligands, such as thf, results in the release of N_2 gas and the formation of a monomeric complex with the formula $[V(RNC(R')NR)_2(thf)_2]$.[676]



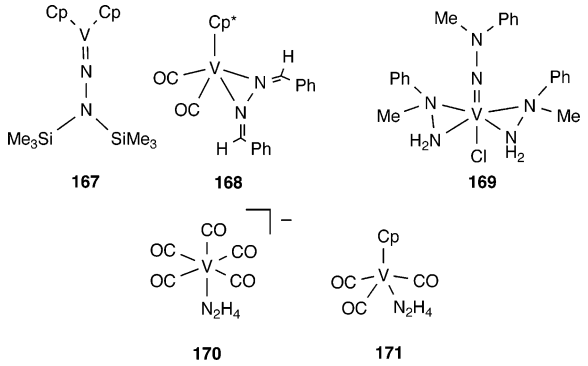
Documenting the precedence for organometallic complexes such as **162** and **163** are important to the goal of developing catalysts that fix N_2. The mononuclear V(III)-complex with (dimethylamino)methylphenyl (**162**) was prepared.[677] The dinuclear precursor complex $[V(Me_3Si)N\{CH_2CH_2N(SiMe_3)\}_2)Cl]_2$ (**164**) was used to generate the doubly nitrido-bridged V(V)−V(V) and V(V)−V(IV) dimers (**163**, **165**, **166**).[678,679]

7.7. Formation of Hydrazine and Other Reduced Compounds

The Nase from *Az. chroococcum* produces NH_3 and trace amounts of N_2H_4 (eq 14).[634] The formation of N_2H_4 prompted investigations into the properties of V(III)-complexes with N_2H_4 and their derivatives.

$$N_2 + 4H^+ + 4e^- \rightarrow N_2H_4 \qquad (14)$$

Substituted hydrazines form dinuclear V(III)-complexes in which the hydrazine bridges the two V-atoms (**164**).[680] In mononuclear complexes **167**−**171**, the two hydrazine groups are generally coordinated head-on in a trans configuration.[680] While most complexes have the hydrazine group coordinating head-on (such as **171**[681]) some are thought to coordinate side-on, **168** and **169**.[682] Although reactivity studies need to be examined for many of these complexes, the organometallic $[V_2N_2(mes)_6]$ (**168**) complex was investigated[683] and found to produce NH_4^+ and/or $N_2H_5^+$ with protic reagents. V−Nase also exhibits an alkyne reductase activity as shown in eq 15.[684,685] Isocyanide reductase activity has also been reported (eq 16).[686]



$$\text{-C}\equiv\text{C-} + 2H^+ + 2e^- \rightarrow \text{-C}=\text{C-} \qquad (15)$$

$$RNC + 6H^+ + 6e^- \rightarrow CH_4 + RNH_2 \qquad (16)$$

8. Additional Focus Areas in Biomimetic Vanadium Chemistry

A variety of enzyme activities have been observed by V-compounds in addition to those described above. In this section, we will briefly review reports on activities of V-compounds as well as describe emerging systems in bioinorganic V-chemistry. We will begin by describing the vanadium peroxidase and catalase activities of V-compounds. Then porphyrin derivatives and their properties and roles followed by the insulin mimetic properties of V-complexes will be discussed. Finally, a few additional areas of interest will be summarized including the interaction of serum proteins with V-compounds, and the molybdenum-free nitrate reductase.

8.1. Peroxidase Activity of Vanadium Compounds

Peroxidases catalyze the oxidation of a variety of substrates using H_2O_2 as an oxidant. The activity has been observed both with VHPOs and vanadium model compounds[6,7,38,47,687] as well as other metal complexes.[688] Although the enzyme can use acyl peracids as an oxidant, the lower efficiency of the enzyme with this agent results in the accumulation of oxidized intermediates in solution.[47,689] In model systems, these donors are quite effective.[162,690,691] Recently, Pecoraro and co-workers suggested that the VHPOs may actually be vanadium peroxidases and that the well-known halogenation reactions may simply reflect the abundance of chloride in the marine environment.[7,38] The VHPO from *As. nodosum* can oxidize pseudohalides such as cyanide[6,47] and thiocyanate.[47,687] The number of substrates converted by this class of enzymes is constantly increasing and is of interest, in part, because such reactions provide information relevant to understanding the role of these enzymes and because of the synthetic utility of the reactions.[7,38,46,146,170,181,691] Electron-rich

substrates that can be accepted by the enzyme are prime candidates for electrophilic oxidation.

The sulfide oxidation reaction has been the subject of many investigations using both enzyme and model compounds as catalysts. The stereoselectivity of this reaction has been a key aspect of these studies, and reactions yielding stereoselective oxidations have now been reported.[38,46,146,162,170,258,264] The oxidation of thiol groups has been implicated as being relevant to the proposed mechanism of the peroxovanadate interaction with insulin receptors to induce the receptor stimulated protein phosphorylation.[7,319] Recently, mononuclear model systems were found to be competent to oxidize sulfides to sulfoxides in the presence of one equivalent of acid.[257,692] Evidence for a hypobromite-like vanadium intermediate, $[VO(OH_2)(OH)(OBr)]^+$, has been obtained using electrospray mass spectrometry on the vandate/H_2O_2/Br^- system.[231] Model compounds such as **28**−**31** did not react with styrene or cyclooctene when tested, presumably because more electron-rich substrates were needed for the reaction to take place.[257,692]

8.2. Catalase Activity of Vanadium Compounds

The Fe-heme containing haloperoxidases catalyze the disproportionation of H_2O_2 into singlet excited-state oxygen;[139] however, the VHPOs can catalyze this reaction only in the presence of Br^- or I^-.[693] This catalase reaction is favored at higher pH values than the haloperoxidase reaction which is favored at lower pH values.[139] Studies with model compounds **28**−**31** in the absence of an organic substrate will generate dioxygen with the addition of a single equivalent of both H_2O_2 and base. The reaction does not occur in acidic solution, presumably due to the formation of HOBr. In organic solvents, the disproportionation of H_2O_2 is stoichiometric; however, sequential addition of H_2O_2, acid, and base can produce one more cycle of the reaction.[7,38,189] Several Schiff base metal complexes with catalase activities are currently under investigation and specifically the Mn complex holds promise as a potential pharmaceutical agent for the treatment of strokes and other life-threatening problems.[694]

8.3. Vanadium Porphyrins

Porphyrins form very stable complexes, in particular with VO^{2+}, and often function as a thermodynamic sink in the biosphere. VO-porphyrins represent a substantial fraction in crude petroleum[695] and coal;[696] a significant byproduct of refining is vanadium sulfide. Synthetic and structural studies tend to focus on V(IV) porphyrin derivatives,[697,698] but a few V(II)[699,700] and V(III)[701] porphyrins structures have been reported. Homo-[702,703] and heterodimers[703] could be observed by EPR spectroscopy at −193 °C. Vanadyl porphyrins provide a convenient model system for the more reactive oxoiron(IV) porphyrinate intermediate in oxygen atom transfers.[704−707] In nonaqueous media, multiple oxidation and/or reduction steps are common,[708−710] and redox reactions take place both in the porphyrin ring and on the metal center. EPR studies show that in the solid vanadium octaethylporphyrin complex, [VO(OEP)], the unpaired electron resides in the metal $b_2(d_{xy})$ orbital. Upon oxidation, an electron is removed from the porphyrin ring rather than from the metal center.[706,707] Reduction with one electron places the electron in a porphyrin orbital rather than in the metal d orbital[711] analogous to the redox processes observed in oxoiron(IV) porphyrinates.[712]

The heme environment in V-containing myoglobin and horseradish peroxidases was synthesized and investigated using EPR spectroscopy.[713] V(IV) porphyrins were characterized by both vibrational and electronic spectroscopy,[714] and ESEEM spectroscopy.[715,716] An anomalously polarized resonance line in the Raman spectrum suggested that V(IV) is located out of the porphyrin plane as is also the case with the Fe(III) ion.[717] As anticipated, the V−O stretching Raman frequency was sensitive to the axial ligand's π-donor characteristics.[718] Changes in Raman spectra of porphyrin cation radicals provided additional insights for heme porphyrin intermediates.[704] EPR spectroscopy indicates that an unpaired vanadium electron resides in a relatively pure d_{xy} orbital. Axial interaction of vanadyltetraphenylporphyrin VO(TPP) with Lewis bases was found to be weak ($K < 2$) and sterically hindered.[719]

V(IV) porphyrin intercalation complexes with DNA were reported, and these complexes are being considered as anticancer agents.[720−722]

8.4. Insulin-Like Effect of Vanadium Compounds

The insulin-like effect of vanadium salts on cells[723,724] and diabetic animals[725−731] has been known since the 1980s. Diabetic patients frequently have both abnormal glucose and lipid metabolism, which can be normalized by treatment with insulin. Although glucose levels in blood and serum were the original methods for monitoring the symptoms of diabetes, the correction of abnormal lipid metabolism in the diabetic state is now also accepted as important in the management of this disease. Recent work in understanding the pathophysiology of diabetes has included studies of both glucose and lipid metabolism.[732] Studies testing compounds in animal model systems[726−731] and in human beings[733−736] show that simple vanadium salts and V-complexes alleviate the symptoms of diabetes. The STZ-induced diabetic Wistar rat is the most common animal model used for evaluating the insulin-like effect of metal complexes. V-compound treated diabetic Wistar rats can be divided into responding and nonresponding groups with respect to the lowering of diabetic hyperglycemia presumably due to genetic variation in the animals.[330,727] Initially, the vanadium salts were believed to be able to substitute for insulin; however, currently their effects are most commonly attributed to enhancing insulin action.[737]

Peroxovanadium(V) complexes were the first group of compounds showing significantly higher activity than the simple inorganic vanadium salts.[174,175,277] These compounds are of particular interest because of their extraordinary (and still unsurpassed) potency in stimulating the phosphorylation of the insulin receptor (IR) in in vitro assays.[174,175,738,739] The effects

of simple vanadium salts and peroxovanadium compounds on protein tyrosine phosphatases and insulin receptor phosphorylation have been useful agents in studies probing the phosphorylated insulin receptor.[319] These studies were important because they demonstrated that hydrolysis of the peroxovanadium compound did not explain the observed effects observed in cells or in animal model systems. A large class of compounds based on V(IV) chelate complexes[726,740] have been extensively studied mainly by the Sakurai and Orvig/McNeill groups, respectively.[64,729,740,741] The effects of bis(picolinato)oxovanadium(IV)[740,742] and the effects of bis(maltolato)-oxovanadium(IV),[727] have been described in great detail and have also been reviewed.[64] In addition, recent reports have documented the effects of certain V(V)-complexes in animals.[66,67,743] One class of V(V)-complexes are the dipicolinate vanadate complexes, and the chemistry of these V-complexes have been examined in detail.[66,67] Another class of V(V)-complexes are formed with hydroxamate ligands, and these compounds have been prepared in situ.[743] These studies counter earlier reports of inactive V(V)-complexes derived from maltol.[728] A few insulin enhancing V(III)-complexes have been reported[68] suggesting that the vanadium oxidation state does not seem to be as critical as previously believed. Reviews describing the effects of a number of V-compounds tested in animals show how the number of compounds is rapidly increasing.[64,737] The insulin mimetic effect of V-complexes has now been reported to extend from V to other metal complexes including Cr,[744] Zn,[745−747] and Co complexes.[748]

Because the insulin signaling system is exceptionally complex, we have yet to understand all of its intricate details. As such, it is not reasonable to expect the action of effective insulin-enhancing agents to be readily understood. Indeed, to date, no single target can explain all the observed insulin-like effects of V-compounds.[323,327,749−751] The insulin-like effect of V-compounds likely involves a protein tyrosine phosphatase.[13,175,751] Specifically, vanadate is generally believed to exert its insulin enhancing effect through competitive inhibition of regulatory protein phosphatases, with a major candidate being phosphatase 1B (pp1B). This phosphatase is the first phosphatase in the insulin regulatory cascade and is particularly sensitive to inhibition by V-compounds. Inhibition of pp1B in a knock-out mouse led to increased insulin sensitivity and obesity resistance[752] and with antisense RNA led to increases in insulin-dependent signaling in the ob/ob mouse.[753] However, the potency of the competitive inhibition of phosphatases by V-compounds depends on the structure of the complex, the oxidation state of the metal ion, and the nature of the phosphatase[13,272,314,316,319,754,755] and differs by several orders of magnitude, allowing for selectivity of V-compounds in vivo. Some V-compounds such as peroxovanadate irreversibly oxidize the catalytic cysteine in protein phosphatases,[319] while simple vanadate salts[319,739] (and presumably $[VO(malto)_2]$) do not undergo similar redox chemistry, which may explain their smaller effect on the autophosphorylation of the IR.[175−177] It is likely that other aspects of regulation remain to be unfolded. For some time, we have been advocating that the failure to document a correlation between phosphatase potency and effect in vivo is because this one variable alone cannot explain the observed insulin-like effects of V-compounds. We are currently conducting a multimetal compound analysis based on a Compound Profile database. In such an analysis, correlations between phosphatase inhibition (as well as other selected factors) and the insulin-enhancement effect of transition metal compounds in Wistar rats on the lowering of elevated cholesterol levels were observed (unpublished).

The biodistribution and transport of compounds into cells are likely to play important roles in the mode of action of the V-compounds. By default, the compounds must cross lipid interfaces in cells to reach their desired target. Studies have been done showing that after the administration of the compound to animals, the compounds will decompose into the metal ion and the ligand after a certain amount of time.[756,757] Since the metal ion and ligand do not remain intact, the hypothesis that the adsorption and transport of the compounds would involve the loss of organic ligands has been put forward.[756,757] The interaction of vanadyl cation with transferrin was characterized in detail several decades ago by Chasteen and co-workers,[36,295] and these studies as well as more recent studies will be described below. The binding of V(IV) to biological ligands, in particular to transferrin, is important because if transferrin would bind the intact or partially intact V-complex, responses to different metal compounds would vary. In the event transferrin could bind V-complexes with different affinities, the ligand could be more than a vehicle to transport the vanadium ion across the membrane. Indeed, recent studies[306] have reported that transferrin abstracts the vanadium from $[VO(malto)_2]$ contrary to earlier studies carried out with $[VO(malto)_2]$[750] and $[VO(acac)_2]$,[758] which reported that species other than the vanadyl cation were bound to the transferrin. A comparison of the transport of $[VO(malto)_2]$, $[VO(acac)_2]$, and vanadate suggests that the two former complexes, when intact, can enter cells through passive diffusion.[759] The transport of these compounds into erythrocytes is not affected by a phosphate anion-transport blocking agent in contrast to the transport of vanadate, which is inhibited.[759] Additional information on the mode of transport of these compounds across membranes and by transferrin is critically linked to when and where the compound falls apart into ligand and metal ion; this will be key to determining how important transport is to the effectiveness of these compounds.

A third mechanism gaining increasing recognition involves effects of the metal complex on the redox regulation of the cellular environment. Cytoplasmic reduction of vanadate by GSH has been recognized since 1980 and has been extensively studied.[760] Cytoplasmic reduction of vanadate to V(IV) can explain why the Na,K ATPase in vivo seemed resistant to inhibition by vanadate. V(IV) is a less potent inhibitor of the Na,K ATPase and when vanadate is reduced to V(IV), it cannot inhibit this enzyme at

attainable in vivo concentrations of V(IV). The coordination complexes that form between V(IV) and GSH have been described by several groups,[300,301,761−764] and the specific species that form are somewhat controversial depending on pH, and the metal-to-ligand ratio.[300,301,761,763,764] Surprisingly, little structural information is available on metal ion-GSH complexes,[765] although one related V(IV)-complex with cysteine methyl ester[766] has been reported. A variety of spectroscopic techniques have been used to examine V-complexes. Depending on the conditions, eight to nine different V(IV) species have been observed by EPR spectroscopy in the presence of 100-fold excess of GSH.[300,301] Because GSH forms even weaker complexes with V(V) than with V(IV) with GSH, and because GSH will undergo oxidation while V(V) is reduced, this reaction is much less understood.

The role of oxidative stress in the etiology of diabetes has recently been proposed to be involved in both the origin of the disease and increasing secondary complications.[767] The role of oxidative stress caused by glucose toxicity and the resulting production of free radicals, especially in the pancreas, has been proposed to be a major cause of the development of insulin resistance in both type 1[768] and type 2 diabetics.[769−771] This is especially important in patients with diabetic complications. Since antioxidant therapy is currently being investigated to decrease the loss of insulin sensitivity[768] it is likely that some of the effects of metal complexes, whether they be beneficial or toxic effects, may be due to redox regulation. In rats with STZ-induced diabetes, vanadate administration was shown to decrease GSH while the levels of GSH reductase and oxidized GSH remained unchanged.[772] In comparison with insulin, vanadate was only partially able to control the impaired antioxidation system of diabetic rats. In the same model system, both vanadate and insulin improved lipid metabolism as monitored by total lipid levels, triglyceride, and lipogenic enzymes.[773] In examining different tissues, vanadate administration was also found to have antioxidant properties when the activities of antioxidant enzymes catalase, superoxide dismutase, and GSH peroxidase were measured.[774] The above results suggest that antioxidant effects of the oral administration of vanadate have been established in diabetic rats and support the hypothesis that changes in cellular GSH metabolism are connected to the insulin-enhancing properties of transition metal complexes.

Current recognition of both the negative and the positive effects of oxidation−reduction reactions in biology has led to the cellular redox state being increasingly investigated. Various examples of protein and metabolism regulation have been described,[775−778] including the proposal that cellular oxidation mediates insulin resistance.[767] Decreased glutathione (GSH) levels have been implicated in the lowered resistance of diabetics to oxidative stress.[779] Complications of diabetes have been correlated with reduced GSH levels, suggesting that antioxidants such as GSH can protect against diabetic complications.[780] The vanadate-stimulated NAD(P)H oxidation[781−783] has been extensively investigated. The possibility that this activity may be linked with the insulin-enhancing redox-related modes of action has not previously been proposed, presumably because superoxide was not yet recognized as an inducer of normal metabolic processes. However, studies have been conducted linking vanadate-induced cell growth regulation to reactive oxygen species.[341] Vanadium-induced Fenton[781,783] and Haber-Weiss chemistry[781,783] has been investigated, but the possibility that these reactions are linked to the insulin-enhancing properties of metal complexes has not previously been proposed. Studies of known and intact coordination complexes combined with a speciation analysis will be necessary in such investigations and are likely to emerge for this and other pathways regulated by the redox state of the cell.

With all the beneficial action of the variety of different V-complexes, the failure to implement these compounds in the treatment of diabetic patients can mainly be attributed to the toxicity of the compounds.[735−737,784] Although the toxic effects are initially manifested as the failure to gain weight and gastric irritation, the fact remains that for the simple salts the therapeutic window is too narrow for further development.[735,736,784] Although it may be possible to dissociate the toxic effects of V-compounds from their beneficial effects, the progress in this area has been complicated by the desire to first understand the mode of action of these compounds. As stated above, diabetes is a multifaceted disease that is likely to involve multiple pathways. These considerations may be further exacerbated by the possibility that some of the beneficial effects of V-compounds may in fact involve reactive oxygen species (ROS), which were recently recognized as important in the normal control of metabolism by inducing certain transcription factors.[785] Studies in cellular systems with a range of compounds are becoming increasingly important as more information on the action of different classes of V-compounds in various cellular systems becomes available (see for example, refs 30, 312, 331−342, 731, and 786).

8.5. Affinity for Transport Proteins: Transferrin and Serum Albumin

The affinity of serum transport proteins, particularly transferrin, became of increasing interest when the therapeutic potential of V-compounds was recognized. Model systems to characterize the interaction of serum proteins with V-compounds continue to be reported and both structural and spectroscopic techniques are used to investigate the suitabilities of these models[787] to the direct studies reported with the protein.[36,788,789] The interaction of vanadyl cation and vanadate with both transferrin and serum albumin was investigated in detail several decades ago by Chasteen and co-workers[36,295] and more recently by others.[789−793] Transferrin has an affinity for vanadyl cation that is at least 10-fold greater than that of serum albumin.[36,790,791,793] Immunoglobins have little if any affinity for vanadyl cation.[750] Serum albumin can associate with up to 20 molecules of V(IV), and the resulting complex will represent a

reservoir of readily accessible vanadium in, for example, infusion solutions.[790,791] Studies using CD spectroscopy suggested a specific interaction between VO^{2+} and Cys-34.[793] FTIR spectroscopy and gel and capillary electrophoresis studies suggested that the VO^{2+} interacted with the histidine nitrogen atom and the terminal α-amino groups whereas vanadate interacted with the ϵ-amino group in Lys residues.[790] At acidic pH serum albumin in the presence of H_2O_2 and VO^{2+} was found to oxidatively deaminate lysine residues to form the corresponding aldehyde functionalities. The reaction was inhibited by EDTA and catalase, and at no point was vanadium as effective as the copper ion in this reaction.[794]

Recent studies have been carried out and demonstrated that vanadium is carried and accumulated in transferrin isolated from animal tissue.[792,795] The V-transferrin complex is sufficiently stable to be able to withstand electrophoresis,[796] HPLC,[797] or mass spectrometry[797] treatment. Although more labile complexes may not have been isolated in such studies, complexes containing vanadium in different oxidation states and with different conformations of the protein have been detected.[797] Few studies have been carried out with V-complexes to answer the question as to whether the abstraction of the vanadium ion from the complex is ligand-dependent.[306,750,758] Increases in ferritin and lactoferrin expression on treatment of respiratory epithelial cells by some metal complexes was reported[798] and confirmed studies supporting the link reported between the activation of transferrin gene expression with tyrosine protein phosphatases.[799]

8.6. Newly Discovered Class of Vanadium-Containing Enzyme

Recently, molybdenum-free nitrate reductase enzymes have been isolated from bacteria that contain vanadium.[800,801] The periplasmic nitrate reductase isolated from *Pseudomonas isachenkovii* bacteria showed a correlation between increasing nitrate reductase activity and vanadium content.[800] The nitrate reductase from *Tv. nitratireducens* contains both vanadium and a heme, possibly in the same subunit.[801] The latter enzyme also exhibits nitrite reductase and haloperoxidase activities. An N-terminal amino acid sequence comparison using Swiss-Prot Bank for this enzyme failed to show a relationship of this enzyme with the molybdenum-containing nitrate reductases, nitrite reductases, and haloperoxidases.[801]

9. Summary

V-compounds and vanadium-containing proteins have shown a wide range of properties and reactivities, some small-molecule based while others provide the needed structure or function in a biomolecule. Various aspects of this topic have been described in more than 100 reviews over the past decade. It was therefore beyond the scope of this review to be comprehensive. Indeed, the readers are referred elsewhere for more information in the various areas, and key reviews are listed to provide the novice reader with access to excellent sources of information in the various mature and developing areas. New areas identified in this review include the new vanadium-binding proteins, vanabins, isolated from the tunicates, which are proposed to be the first vanadium transport proteins, and represent only the third class of proteins (VHPO, VNase and vanabins) that bind vanadium naturally. The current developments in studies demonstrating haloperoxidase activities from other enzymes to which vanadate was added, and the recognition that this may be a general feature of hydrolytic enzymes which may exhibit similar activities upon binding a metal ion open up new areas in chemistry and biology. Since many enzymes are found to have dual functions in biological systems, the fact that the addition of vanadium (and perhaps other metal ions) can change the action of a protein may have far-reaching implications for how vanadium affects biological systems.

Of particular relevance to this review is the observation that V-compounds have been reported to exhibit increasing numbers of enzyme activities. The combined range of vanadium bioactivity is vast. The vanadate−phosphate analogy places vanadate in a central position to interact effectively with phosphorylases. Indeed, enzymologists and bioinorganic chemists have exploited this analogy for decades, and it continues to give results in the structural arena that are important to biologists. Although vanadate may not be a perfect transition state analogue and may not be able to provide the entire binding affinity anticipated for an ideal analogue, very few alternatives exist, and certainly not any with similar high binding affinities. However, other activities of V-complexes are beginning to play a role and show some selectivities that are very promising such as that observed in the photochemically induced cleavage of myosin and F_1 ATPase at one specific amino acid residue by vanadate. Other activities, such as the peroxidase and haloperoxidase activity exhibited by amavadine, provide important information to chemists. If amavadine is truly kinetically inert, these activities would imply outer sphere reactions and would thus serve as a new prototype of reactivities exerted by V-complexes. The ability of V-complexes to act as peroxidases, catalases, and nitrogenases is very important, and suggest, not surprisingly, that many reactions of V-compounds include redox processes. Given that many of the compounds able to model enzyme reactions, are under consideration as therapeutic agents, can serve as tools in biological investigations, and are exhibiting new types of reactivities, investigations into vanadium chemistry are likely to grow significantly in the future.

10. Acknowledgments

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11. List of Abbreviations

A. ceratodes	*Ascidia ceratodes* tunicate (sea squirt)
Az. chroococcum	*Azotobacter chroococcum* nitrogen-fixing bacterium

A. gemmata	*Ascidia gemmata* tunicate (sea squirt)
Am. muscaria	*Amanita muscaria* mushroom
As. nodosum	*Ascophyllum nodosum* alga
A. sydneiensis samea	*Ascidia sydneiensis samea* tunicate (sea squirt)
Ar. thaliana	*Arabidopsis thaliana* flowering plant
Az. vinelandii	*Azotobacter vinelandii* nitrogen-fixing bacterium
acac	acetylacetonante
ADP	adenosine 5′-diphosphate
ADPV	adenosine 5′-diphosphate vanadate
AMP	adenosine 5′-monophosphate
AMPV	adenosine 5′-monophosphate vanadate
AMV	adenosine 5′-monovanadate
ATP	adenosine 5′-triphosphate
ATPase	adenosine triphosphatase
B. stearothermophilus	*Bacillus stearothermophilus* bacterium
Bicine	*N*,*N*-bis(2-hydroxyethyl)glycine
BMOV	bis(maltolato)oxovanadium(IV)
bipy	2,2′-bipyridyl
bispicen	*N*,*N*′-bis(2-pyridylmethyl)ethylenediamine
Bpz	tris(1-pyrazolyl)borate
Ca. fumago	*Caldariomyces fumago* fungus
C. inaequalis	*Curvularia inaequalis* fungus
Co. officinalis	*Corallina officinalis* alga
Co. pilulifera	*Corallina pilulifera* alga
Ch. vulgaris	*Chlorella vulgaris* alga
cat	catechol
CD45	protein tyrosine phosphatase CD45
DOPA	3-(3,4-dihydroxyphenyl)alanine
DFT	density functional theory
dmso	*N*,*N*-dimethylsulfoxide
3,5-dtbc	3,5-di-*tert*-butylcatechol
En. cloacae P99	*Enterobacter cloacae* P99 bacterium
E. coli	*Escherichia coli* bacterium
EPR	electron paramagnetic resonance
ESEEM	electron spin−echo envelope modulation
EXAFS	extended X-ray absorption fine structure
FAD	flavine−adenine nucleotide
FeMoco	iron−molybdenum cofactor
H_3hidpa	*S*,*S*-2,2′-hydroxyiminodipropionic acid
heida	*N*-(2-hydroxyethyl)iminodiacetate
HSALIMH	[4−2-(salicylideneaminato)ethyl]imidazole
LAR	leucocyte antigen related protein tyrosine phosphatase
M. manhattensis	*Molgula manhattensis* tunicate (sea squirt)
malto	maltolato
MCDD	2-chloro-5,5-dimethyl-1,3-cyclohexadione
meida	*N*-methyliminodiacetic acid
mes	mesitylene
NAD	β-nicotine adenine dinucleotide, oxidized form
NADH	β-nicotine adenine dinucleotide, reduced form
NADP	β-nicotine adenine dinucleotide phosphate
NADV	β-nicotine adenine dinucleotide vanadate
NMR	nuclear magnetic resonance
ox	oxalate
OEP	octaethylporphyrin
P. aeruginosa	*Pseudomonas aeruginosa* Gram-negative bacterium
P. isachenkovii	*Pseudomonas isachenkovii* bacterium
Ph. mammillata	*Phallousia mammillata* tunicate (sea squirt)
Ph. nigra	*Phallusia nigra* tunicate (sea squirt)
Ps. occelata	*Pseudopotamilla occelata* fan worm
pp1B	protein tyrosine phosphatase 1B
PTK	protein tyrosine kinase
PTP	protein tyrosine phosphatase
py	pyridine
^{BrNH2}pyg	*N*-(2-pyridylmethyl-3-bromo-6-amino)-iminodiacetic acid
quin	quinoline
shi	salicylhydroximate
STZ	streptozotocin
terpy	terpyridine
thf	tetrahydrofuran
tpen	*N*,*N*,*N*′,*N*′-tetrakis(2-pyridylmethyl)-ethylenediamine
TPP	*meso*-tetraphenylporphyrin
TS	transition state
Tv. nitratireducens	*Thioalkalivibrio nitratireducens* nitrate reducing bacterium
V-compounds	vanadium compounds
V-complexes	vanadium complexes
VAP	vanadium-associated proteins
VBPO	vanadium bromoperoxidase
VCPO	vanadium chloroperoxidase
V−P	vanadate−phosphate analogy
VHPO	vanadium haloperoxidase
VNase	vanadium nitrogenase
XANES	X-ray absorption near edge structure

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