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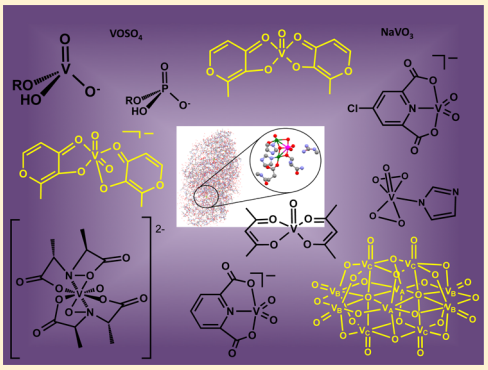
Antidiabetic, Chemical, and Physical Properties of Organic Vanadates as Presumed Transition-State Inhibitors for Phosphatases

Debbie C. Crans*

Department of Chemistry and Cell and Molecular Biology Program, Colorado State University, 1301 Center Avenue, Fort Collins, Colorado 80523, United States

ABSTRACT: Studies of antidiabetic vanadium compounds, specifically the organic vanadate esters, are reviewed with regard to their chemistry and biological properties. The compounds are described from the perspective of how the fundamental chemistry and properties of organic vanadate esters impact their effects as inhibitors for phosphatases based on the structural information obtained from vanadium−phosphatase complexes. Vanadium compounds have been reported to have antidiabetic properties for more than a century. The structures and properties of organic vanadate complexes are reviewed, and the potency of such vanadium coordination complexes as antidiabetic agents is described. Because such compounds form spontaneously in aqueous environments, the reactions with most components in any assay or cellular environment has potential to be important and should be considered. Generally, the active form of vanadium remains elusive, although studies have been reported of a number of promising vanadium compounds. The description of the antidiabetic properties of vanadium compounds is described here in the context of recent characterization of vanadate−phosphatase protein structures by data mining. Organic vanadate ester compounds are generally four coordinate or five coordinate with the former being substrate analogues and the latter being transition-state analogue inhibitors. These studies demonstrated a framework for characterization of five-coordinate trigonal bipyramidal vanadium inhibitors by comparison with the reported vanadium−protein phosphatase complexes. The binding of the vanadium to the phosphatases is either as a five-coordinate exploded transition-state analogue or as a high energy intermediate, respectively. Even if potency as an inhibitor requires trigonal bipyramidal geometry of the vanadium when bound to the protein, such geometry can be achieved upon binding from compounds with other geometries. Desirable properties of ligands are identified and analyzed. Ligand interactions, as reported in one peptidic substrate, are favorable so that complementarity between phosphatase and coordinating ligand to the vanadium can be established resulting in a dramatic enhancement of the inhibitory potency. These considerations point to a frameshift in ligand design for vanadium complexes as phosphatase inhibitors and are consistent with other small molecule having much lower affinities. Combined, these studies do suggest that if effective delivery of potentially active antidiabetic compound such a the organic vanadate peptidic substrate was possible the toxicity problems currently reported for the salts and some of the complexes may be alleviated and dramatic enhancement of antidiabetic vanadium compounds may result.



1. INTRODUCTION

The antidiabetic effects of vanadium compounds has been known for more than a century;[1] however, the epidemic increase in the prevalence of diabetes has made exploration of less convential drugs more attractive.[2−38] This Perspective reviews the antidiabetic activities of simple and complex vanadium compounds. The focus in this review is on studies of structures and properties of organic vanadium complexes to understand how structural differences in inhibitors for phosphatase translate to functional differences in alleviating the symptons of diabetes. This involves consideration of the coordination geometry of the vanadium compounds and associating the structure of the compounds to their biological effects as they pertain to diabetes. An excellent example is that seen for organic vanadates, which when four coordinate are generally analogues of organic phosphates and substrates for enzymes converting organic phosphates.[9,21−24] In contrast, when the vanadium is five coordinate they are transition-state analogues of phosphoester hydrolytic enzymes such as phosphatases.[9,24,25] Based on these considerations, we point to this simple change in the coordination geometry of the vanadium which will dramatically change how the vanadium compounds will act. In the following, we examine a range of organic ligands with the objective of exploring how ligands can change the action of the organic vanadate.

Although many salts and coordination complexes were investigated as antidiabetic agents in various animal model systems, only a few of these compounds were selected for studies in human beings, that is, studies in clinical trials (more description below).[26−30] The vanadium compounds that have been in clinical trials (phases I and II) are shown in Figure 1. The first studies were done with the simple salts metavanadate and vanadyl sulfate. The studies with vanadate and vanadyl salts

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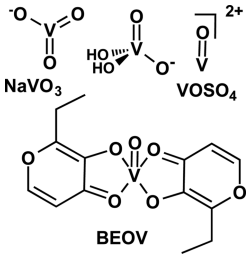

Figure 1. Vanadium compounds that have been in clinical (more description below) trials: sodium metavanadate (shown both as $NaVO_3$ and the solution form NaH_2VO_4), vanadyl sulfate, and bis(ethylato)oxovanadium(IV) abbreviated BEOV.[3,26−29,31]

originated from an National Institute of Health RFA from the NIH for testing these drugs in human beings, as so few promising compounds were available to treat diabetes.[26−31] The early studies on diabetic subjects were similar to what would occur in a phase II clinical trial but were not carried out as part of an effort to market the drug. Phase I type clinical trials on healthy subjects were not required for the salts in part because no toxic effects had been reported for years use of several vanadium supplements even though these salts have been and continue to be available commercially as diet supplements. Permission to investigate the effects of vanadate and vanadyl sulfate on diabetic human beings was therefore readily obtained, and the clinical studies equivalent to phase II studies were carried out.[5,26−29,31] Although animal studies had been carried out using other forms of vanadate, sodium metavanadate was generally selected because it forms solutions at neutral pH whereas orthovanadate forms highly basic solutions. In the most recent clinical study involving 1598 Chinese human beings, the nature of the vanadium salt used for treatment was not specified, although the plasma vanadium levels in newly diagnosed cases of diabetes were lower than in the healthy control group of human beings.[30] One organic vanadium complex, bis(ethylato)oxovanadium(IV) (BEOV) also completed phase II clinical trials.[3] Importantly, no toxic effects were observed in these studies. However, the effect of BEOV on the diabetic patients was variable, and the clinical trials were abandoned because the compound went off patent Sept. 30, 2011.[3]

Many more compounds are and have been investigated as potential agents for treatment of diabetes[2−20,32−37] and also for appetite suppressant.[45] Currently, the effect of vanadium as an anticancer agent is being increasingly investigated by several groups.[33,38−44] Progress in the area of antidiabetic agents has been most effective when both chemical and biomedical studies has been done in concert, and this is evident in the contributions by several teams with both chemists and medical scientists.[5,6,13,17,33,45] Therefore, this Perspective will describe a significant amount of fundamental vanadium chemistry, biochemistry and pharmacology. This contribution is prepared as a result of a 2015 Cope Scholar award presentation on the studies of the structures and properties of organic vanadium complexes and applying these principles to understand the impact of different ligands on antidiabetic activity. The present review will describe the chemistry of vanadium compounds in the context of inhibitory effects of organic vanadates for protein tyrosine phosphatases and illustrate how some of the vanadium compounds can be exceedingly potent inhibitors for protein tyrosine phosphatases.

2. ANTIDIABETIC VANADIUM SALTS AND COMPOUNDS

The early studies were first done with simple salts, Figure 1, then studies were reported with vanadium(IV) coordination complexes[4,6,13,16,17,19] and peroxovanadium(V) compounds,[46−48] Figure 2. More recently, studies were reported

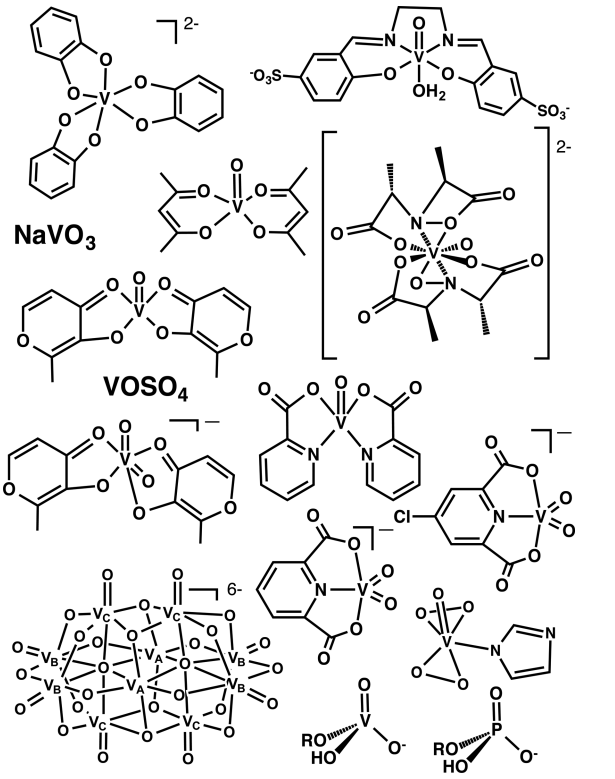

Figure 2. Representative vanadium compounds from the different classes of vanadium insulin-enhancing compounds in addition to those subjected to clinical trials demonstrating in animal studies their ability to lower elevated blood glucose levels in diabetic subjects.[6−11,13,14,16−20] A summary of compounds including, from the top left, a nonoxovanadium compound, $NaVO_3$, $VO(acac)_2$, BMOV (also abbreviated $VO(malto)_2$), $VOSO_4$, oxidized BMOV, and decavanadate and, from the top right, a Schiff base derivative, amavadin, bis-picolinatooxovanadium(IV), 4-chlorodipicolinatodioxovanadium(V), dipicolinatodioxovanadium(V), a peroxovanadium complex, and a general formula for an organic vanadate ester and an organic phosphate ester.

with vanadium(V) coordination complexes,[6,13,16,17,19,20] vanadium(III) complexes[4,49,50] and larger oxometalates,[51−54] Figure 2. Indeed, the ability of vanadium to lower elevated blood glucose and lipid levels is unique among antidiabetic agents: both vanadium salts and complexes lower only the elevated blood glucose levels of diabetic animals rather than normal blood glucose levels. This makes vanadium compounds as antidiabetic agents remarkably safe for treated subjects because the problem of hypoglycemic episodes is less of a concern.[6]

Although the initial studies were done on simple salts, it quickly became clear that the organic vanadium derivatives, that is, a range of different coordination complexes, had potential to surpass the simple salts. Indeed, for a while the peroxovanadium complexes took center stage,[46−48,55] because of their ability to dramatically enhance the observed biological

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responses of the simple salts in *in vitro* cell studies documenting activation of the insulin receptor phosphorylation. Although most researchers recognized that organic ligands improved the effects of the simple salts,[56−58] studies were still carried forward with the simple salts in clinical trials, simply because the documentation of effects in animal studies were needed before it was possible to proceed with a compound in clinical trials.[26−28,30]

Before a compound can be sold as a therapeutic agent, it must undergo human studies referred to as "clinical trials". Clinical trials are a series of studies that must be done before a drug can be marketed, and these trials are divided into phases I−IV. Phase I involves testing on a small number of healthy individuals to see if there are any immediate side effects, and then phase II involves testing on a small number of patients.[26] Phase III expands to a larger number of patients being treated by the drug, and finally, in phase IV, patients are monitored to see if there are any long-term side effects associated with the drug. At this point, two simple salts and one organic vanadium coordination compound had completed phase II clinical trials. Importantly, however, for recognizing the potential of developing compounds, the vanadium coordination complex that completed clinical trials, BEOV, is no longer protected by its patent.[2−4,59] The related compound for which many studies have been reported, bis(maltolato)oxovanadium(V) BMOV or $VO[malto]_2$, remains a key standard to which other new and known antidiabetic compounds are compared because the effects of BEOV are very similar to those of BMOV.[2−4,59] Not as many studies have been reported with BEOV, and BMOV continues to be the compound new systems are compared to.[59] At the time the initial discoveries were made for insulin-enhancing effects, my group was involved in the studies of vanadium(V) compounds, their structure, and both their chemical and biological properties and the phosphate−vanadate analogy. Our studies therefore naturally provided important information on the fundamental properties of vanadium compounds and their potential action.

Vanadium as an early first-row transition-metal ion has similar properties with adjacent metal ions such as chromium[19] and also with second- and third-row elements like molybdenum and tungsten, all of which have been reported to have antidiabetic properties and inhibit phosphatases.[54,60,61] Indeed, it is remarkable that a small transition-metal ion in the form of simple salts and complexes can enhance the function of a 51 amino acid long peptide.[29] Throughout the last three decades, many studies have been reported with many other vanadium compounds, characterizing the fundamental properties of these vanadium complexes, testing compounds as potential antidiabetic agents, and documenting their biological properties.[2−20,62] The literature used both the terms insulin mimetic and insulin enhancing to describe the antidiabetic action of vanadium compounds.[29] A drug can be described as a functionally insulin mimetic when the drug replaces insulin, while an insulin-enhancing drug needs a small amount of insulin present to be effective. When the initial biological effects of simple vanadium salt were reported, the vanadium compounds were referred to as insulin mimetics because the vanadium was believed to replace insulin. This perception was developed because the insulin level in diabetic rats was lower than that of healthy rats, and so it was believed that the vanadium was replacing the absent insulin. However, when vanadium was tested in the genetic diabetes model of the BB rat (a rat model of human Type 1 diabetes), vanadium in the absence of insulin was not sufficient to keep these animals alive. Furthermore, when vanadium was given to these animals with insulin, less insulin was needed.[29] The vanadium compounds are therefore better classified as insulin-enhancing. Not all the different vanadium compounds have been investigated in this manner, but results with those that have been investigated suggest that the vanadate compounds are insulin-enhancing and not insulin-mimetic, and the term insulin-enhancing has been generally adopted as a better description than insulin mimetic for vanadium compounds.

Vanadium compounds have many modes of action.[6,8,12,24,33,47,52,58,59,63] Indeed, as described in detail here, the mode of action is linked to the specific species that is formed: that is, whether the vanadium is present as an anion or cation, as a salt or a complex, and in which oxidation state.[33,63,64] Because of the range of species that can form under physiological conditions, it is nontrivial to determine what species is active in a biological system. Much information has been obtained by correlating chemical studies with *in vitro*, *ex vivo*, and *in vivo* studies. As a result, we know that vanadium compounds perform several activities that are likely to be important in biological systems. Specifically, vanadium compounds are known to stimulate glucose uptake possibly in part by sensitizing insulin,[59] to inhibit phosphatases and other phosphorylases,[12,25,43,51,55,65−78] to induce formation of reactive oxygen species (ROS),[6,50,70,79] and to bind to transferrin.[33,80−82] By understanding the properties of vanadium compound these modes of action this will improve the studies with and development of inhibitors for these systems. All three modes of action are currently being investigated by several groups of researchers in the field. The ability of vanadium to, for example, inhibit phosphatases and other phosphorylases such as Na^+, K^+ ATPases and Ca^{2+} ATPase may be important in a biological system.[7,60,71,77,83,84] There are many phosphatases and phosphorylases that are inhibited by vanadate, so some people worry that vanadium compounds are too unselective because it is difficult to sort out what mode of action is most important. However, animal studies have shown that there is selectivity, and we point out that that such selectivity can be achieved by differences in affinities of the compounds for the different enzymes as well as by access and compartmentalization. Therefore, understanding of transport of the vanadium compounds is critical, whether by diffusion into and out of organelles in the cell or organs in the animal. Indeed, pharmacokinetic studies and distribution studies are sensitive to such matters, although limited information is available in this area. With such complexity, it is not surprising that the question remains which salt and/or organic vanadium species are active in the *in vivo* living being.[5]

In this Perspective, we describe the properties of vanadium compounds in the context of exploring one major mode of action of antidiabetic vanadium compounds: namely, their ability to inhibit protein tyrosine phosphatases. We furthermore analyze many different vanadium compounds subjected to animal studies and their potential as phosphatse inhibitors and compare it with one structurally characterized potent inhibitor of PTP1B, a peptido vanadate ester.

3. VANADIUM COMPOUNDS ARE INHIBITORS OF PHOSPHATASES

Phosphatases are a class of enzymes that remove phosphate groups from a biomolecule.[77,85−87] The substrate for phosphatases range from small metabolites such as sugar

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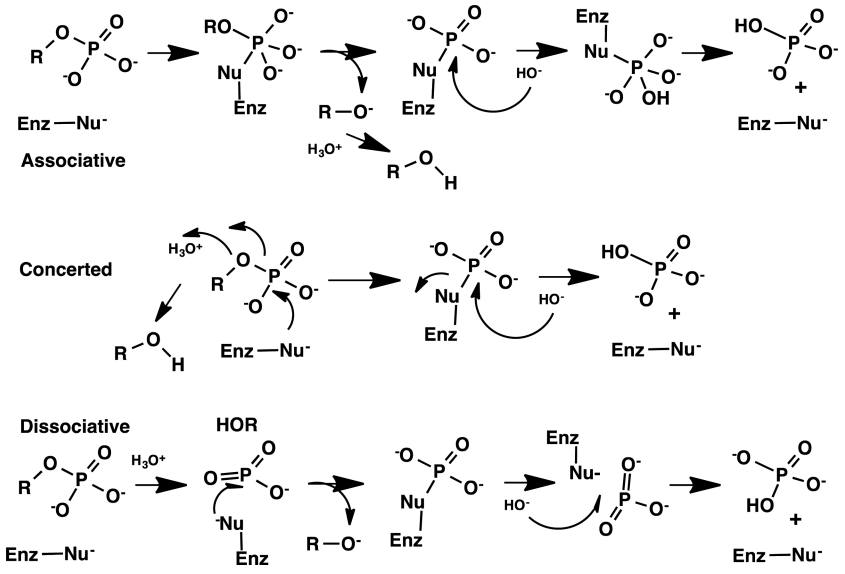

Figure 3. Limiting associative (top) and dissociative (bottom) mechanisms of hydrolysis of a phosphate ester dianions. Only depicted is the concerted reaction from four-coordinate reactant to five-coordinate transition state and to four-coordinate phosphoenzyme intermediate followed by formation of five-coordinate transition state forming four-coordinate product, which is an alternative mechanism used by PTP1B.

phosphates to large proteins, like the phosphorylated insulin receptor. Phosphatases can be very specific as in the case of protein phosphatases, or much more general and accept many substrates as in the case of alkaline phosphatases.[87,88] Phosphatases constitute a very diverse group of enzymes, many of them membrane associated. Substrate and location in the cell are important to their mode of action.[85−87] The limiting phosphate hydrolysis reactions are dissociative or associative mechanisms; however, the PTP1B is known to go by a concerted exploded mechanism in which the bonds between the vanadium and oxygen atoms are sufficiently long to mimic bond breaking in line with what would be expected in a transition state, Figure 3.[76,87,88] In the associative mechanism, the enzyme nucleophile attacks the substrate organic phosphate and forms a five-coordinate transition state analogue. In the concerted exploded transition-state mechanism, the organic phosphate bond begins to break while the enzyme nucleophile attacks to form the five-coordinate-exploded transition state. It has been long recognized that vanadium compounds inhibit all phosphatases, including those that are not described as going by an associative mechanism.[60,84,89−91] It was recently suggested that the geometry of the active site of PTP1B is complementary to the larger vanadate and the longer V═O bond simply matches the space in the active site, making the "exploded transition state" gain stabilization by multiple H-bonding interactions.[92]

The question of whether the observed antidiabetic effects are all attributed to inhibition of phosphatases remains a currently ongoing debate. Protein Tyrosine Phosphatase 1B[93−97] is often considered to be a major contributor to the observed insulin-enhancing effects of vanadium compounds, and the inhibition of solely PTP1B would cause a very specific phenotype. The phenotype of a rat with a knockout of PTP1B does not have the same phenotype as a diabetic rat treated with vanadium compounds. If the vanadium is inducing antidiabetic effects solely due to inhibition of PTP1B, but it is a nonspecific inhibitor, it is expected that the rat with a PTP1B knockout would have a different phenotype as compared to a diabetic rat treated with vanadium compounds. Therefore, it is possible that nonspecific inhibition by vanadium has its antidiabetic effects, not by the range of phosphatases inhibited, but by the more potent inhibition of PTP1B. Furthermore, the inhibition of phosphatases are further complicated by the redox state of the vanadium, ROS, and the type of cells investigated.[6,50,70] In summary, it is possible that the antidiabetic effects are due to the inhibition of PTP1B, the redox effects, ROS, and inhibition of other enzymes such as phosphatases and phosphorylates.

There have been a large number of studies showing that vanadium salts and compounds inhibit phosphatases.[20,43,47,51,55,65−67,70,72,75,98−101] Most of these studies have been enzymatic studies, and the large majority of these studies measure inhibition in a comparative manner, with no attempt to demonstrate that the vanadium compounds are reversible competitive inhibitors.[4,25,43,51,66,77,89−91,98,102] Although such studies provide important insights into the immediate problem the authors are investigating, that is, measuring the inhibition of phosphatases by vanadium compounds, they are more difficult to compare to other studies because competitive inhibitors are not readily compared to inhibitors acting by different mechanisms.

The inhibiting species can be determined by correlating the observed inhibition with concentration of a particular vanadium species.[67] Vanadate in the form of monomeric vanadate is generally the form of vanadium that inhibits the phosphatase.[66] However, for human prostatic acid phosphatase, the vanadate dimer was reported to be the inhibiting species at pH 5.5, Figure 4.[67] Indeed, the apparent inhibition by the vanadate dimer changed to inhibition by the vanadate monomer as the pH of the assay increased for the human prostatic acid phosphatase, documenting a dramatic dependence of the active site structure on pH and presumably the presence of a residue that when protonated bound vanadate differently in the active site.

The studies with the protein tyrosine phosphatase from *Yersinia enterocolitica* were extended to include characterization by X-ray crystallography, including studies of a mutant enzyme W354F PTPase that binds vanadate dimer in the active site.[60] Adducts of vanadium phosphatases have also been charac-

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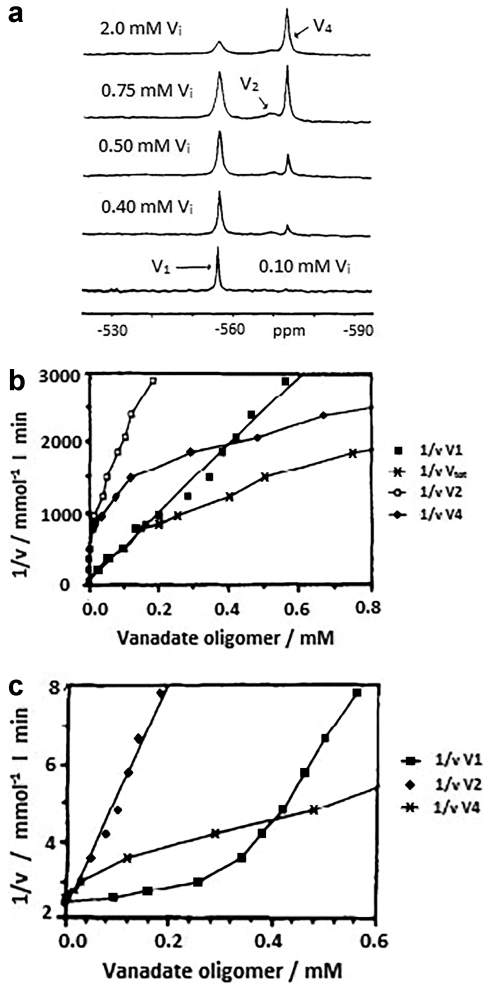

Figure 4. (a) Speciation by ^{51}V NMR spectroscopy of the assay solution documenting the presence of vanadate monomer (V_1), vanadate dimer (V_2), and vanadate tetramer (V_4); (b) linear correlation between vanadate monomer (V_1) and observed inhibition from wheat germ acid phosphatase at pH 5.5 in 0.2 M acetate; (c) the linear correlation between vanadate dimer (V_2) and inhibition of the human acid phosphatase at pH 5.5 in 0.2 M acetate. Adapted with permission from ref 67. 1989 Elsevier.

terized, with an elegant series of studies of vanadium hydroxylamide complexes with protein phosphatases.[20] Kinetic studies combined with NMR and computational work demonstrated how tight these adducts bound in the active site. Finally, it is important to mention that studies have also been done characterizing adducts between vanadate and the PTP1B and in such a manner document the interaction between peroxovanadate and the HS group in the protein phosphatase.[55]

After administration of a vanadium compound, all phosphatases and phosphorylases present are potential targets for the vanadium and contribute to the observed antidiabetic effects.[77] However, it is likely that the enzymes that are most potently inhibited by the vanadium compounds are regulatory enzymes and one such enzyme is the protein tyrosine phosphatase 1B, which is involved in the insulin-signaling pathway. The active site of the PTP1B phosphatase, previously mentioned, is particularly well characterized, and a series of X-ray structures of this protein and a range of mutants have been reported, several of which contain the vanadium in the active site.[60,92] The catalytic cycle has been described in detail using the assistance of two vanadium−PTP1B adducts showing the details of the first exploded transition state (TSA1) in which the vanadium stabilizes the protein and substrate. The second exploded transition state (TSA2) between the phospho-enzyme intermediate and the free enzyme show similar complex H-bonding network in the active site documenting how finely tuned these structures are, Figure 5. Both of these structures nicely illustrate the case in which an enzyme undergoing a concerted hydrolysis reaction is stabilized by a vanadate (which generally stabilize associative mechanisms with five-coordinate transition state structures).[78] In the following, we describe the chemistry and biochemistry of vanadate with a range of ligands all relevant to various aspects of the antidiabetic effects of vanadium salts and complexes.

4. INTERACTION OF VANADATE WITH BUFFERS, CELLULAR COMPONENTS, AND METABOLITES

Because studies of any organic vanadates are very pH sensitive, it is important to consider the conditions under which studies are done. As a result, studies of aqueous vanadium(V) compounds does require knowledge of the fundamental chemistry of vanadate in aqueous solutions. Although previous reviews were done describing the work we and others carried out in this regard, we are here also including some of these studies to illustrate to the reader the properties of these systems. Furthermore, many of these reactions are between an

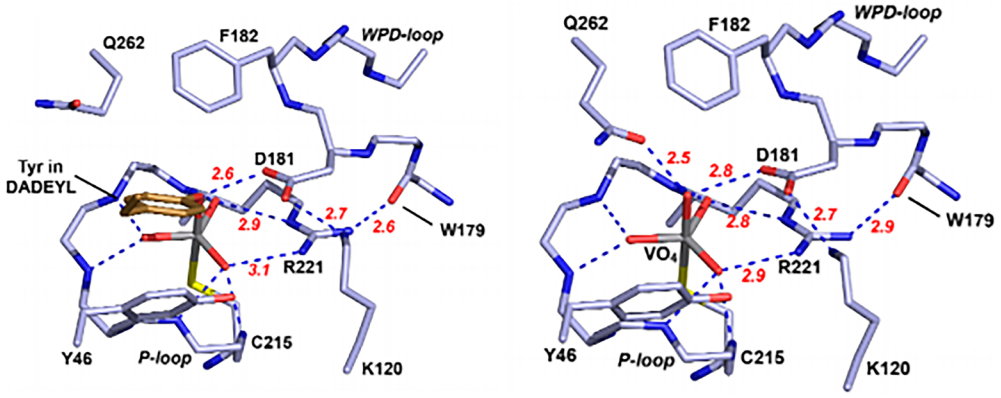

Figure 5. TSA1 (left) and TSA2 (right) and the extensive H bonding network observed for the PTP1B protein bound to the vanadate-DADEYL peptide substrate and vanadate, respectively.[60]

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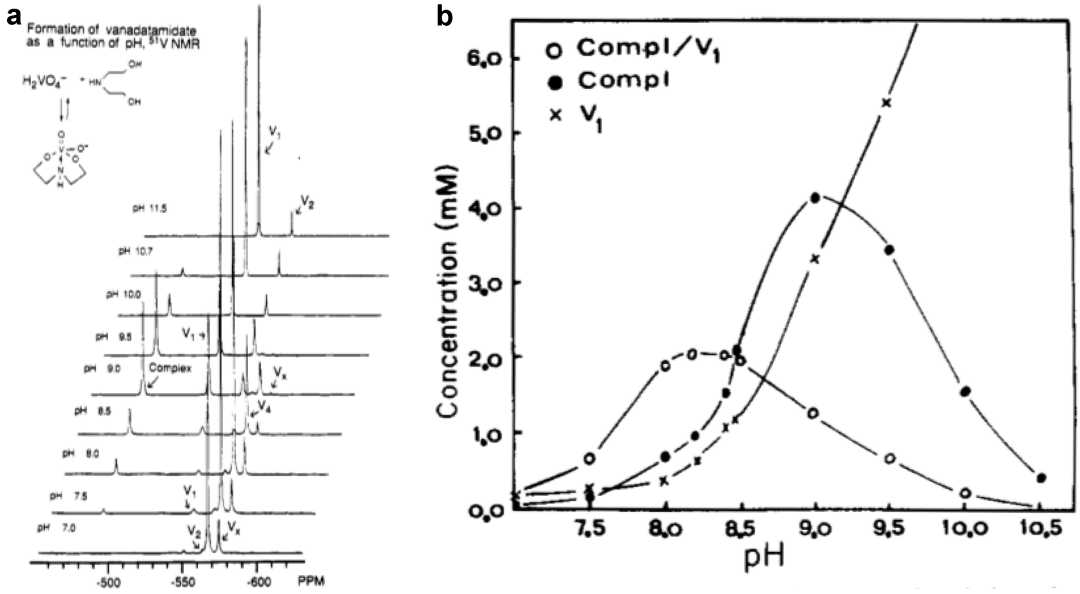

Figure 6. ^{51}V NMR spectra resulting in the measurement of the vanadium(V) complex (a) and the bell curve stability of formation of the complex that formed between vanadate and diethanolamine concentration, V1 concentration and the ratio of concentrations of complex and V1 as a function of pH (b).[104]

alcohol and vanadate which constitute the ester formation. Although a detailed understanding of the chemistry may not be needed for application of vanadium as a probe, these side reactions could potentially cause problems.

4.1. Simple Buffers: Diethanolamine and Triethanolamine. Vanadate is a very reactive species that undergoes reactions with cationic, neutral, and anionic ligands.[9,103] Some common buffers were investigated for their ability to interact with vanadium in the form of vanadate.[24,58,63,66,72,103,104] Indeed, together with my first graduate student, the late Paul Shin, I investigated the vanadium complexes that formed with diethanolamine and triethanolamine in our first publication.[104] The vanadium(V) complex for diethanolamine as investigated by ^{51}V NMR spectroscopy demonstrates that the complex forms in the presence of several oxovanadate species, Figure, 6a. These oxovanadates were found to contain one, two, four, and five vanadium atoms, and their stability was strongly dependent on pH but also on concentration, ionic strength, and other species in solution. In Figure 6b, we show the bell curved stability of the vanadium(V) complex that forms in solution as a function of pH (b). Indeed, observation of the vanadium(V) complexes was readily done using ^{51}V NMR spectroscopy because absorption spectroscopy was not as able to distinguish formation of different oligomeric species.[104] The initial work in this area thus required multiple studies to characterize buffer interactions with vanadate to investigate the nature of the species that formed in order to be able to interpret the results that would be forthcoming.[104−106] As described in this section, this included detailed studies probing the manner in which the V(V) bound to the ligand. Sections 4.1 and 4.2 show that variabilities are observed because of the oxometalates in solution and the fact that these can undergo reactions as well.

$$H_2VO_4^- + H_2dea \rightleftharpoons [VO_2dea]^- + 2H_2O \quad (1)$$

$$HVO_4^{2-} + H_2dea \rightleftharpoons [VO_2dea]^- + H_2O + OH^- \quad (2)$$

Because several commonly used buffers were interacting strongly with vanadate, we continued the characterization of the properties of vanadate coordinated to multidentate ligands in general.[9,24,66,103−112] In multiple reports, vanadium complexes were described with respect to their stability, their solution- and solid-state structure, and eventually the kinetics of the complex formation. In the case of the simple diethanolamine, the most stable complex formed was a multidentate V(V) complex that contained vanadate and the coordination of the buffer ligand in a tridentate manner, $[VO_2dea]^-$ shown in (1) at pH 7.0 when the major monomeric form of the vanadate is $H_2VO_4^-$.[104] As the pH increased, the source of the V(V) became HVO_4^{2-}. However, the product with H_2dea remained $[VO_2dea]^-$, and the additional product was a molecule of H_2O and OH^- as shown in eq 2.

The structure of these simple V(V) complexes were presumed in this early study; however, later in studies with triethanolamine (tea) they were described in detail in solution by spectroscopic studies.[111] Specifically, both H_3tea and the triisopropanolamine (H_3tpa) were investigated by multinuclear NMR spectroscopy including ^{1}H, ^{13}C, and ^{51}V NMR spectroscopy. These studies showed that the H_3tea and H_3tpa coordinated in a tridentate manner to the vanadium with one of the arms remaining uncoordinated. Because that there were two possible structures for such an adduct, ^{17}O NMR spectroscopy was carried out to characterize the species in solution. Two different 1:1 signals were observed by ^{17}O NMR spectroscopy the structure with the VO_2 group in the apical plane was ruled out (shown in Figure 7b), yielding the structure shown below in Figure 7a for the aqueous species.[111]

These studies were accompanied by X-ray crystallogprahic studies of a complex formed between V(V) and H_3tea and characterized in the solid state. Using a solution of $VO(OMe)_3$ and H_3tea in methanol, a complex was isolated and characterized in the solid state to be [VO(tea)], Figure 7c,[111] which is a different structure from the one that formed in aqueous solution shown in Figure 7a. Similar reactions could be carried out if different alcohols (methanol, ethanol, 2-propanol, and *tert*-butyl alcohol) were used.[111] Indeed, the complex formed in methanol, which yielded an X-ray structure that had

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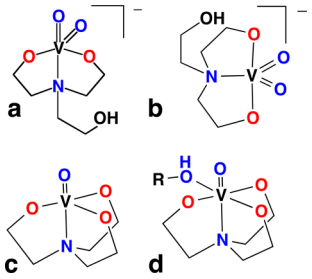

Figure 7. Aqueous solution structure of the $[VO_2(Htea]^-$ complex (a); other possible solution structure (b); solid-state and organic solution structure of the [VO(tea] complex (c); presumed structure for the alcohol adduct [VO(tea) (HOR)] (d).[111]

the H_3tea ligand coordinated in a tetradentate manner to the vanadium.[111] This complex thus contained no free ethanolamine arm in contrast to the complex formed in aqueous solution from vanadate ($H_2VO_4^-$ or HVO_4^{2-}) and H_3tea. Dissolution of the [VO(tea)] in alcohols, in addition to the main complex formed, also formed a second minor species which appears to be a six-coordinate adduct of the alcohol to the vanadium complex, called [VO(tea) (HOR)], Figure 7d.[111] Although this is a minor complex and it was not characterized in detail structurally, the direct coordination to the V(V) atom was surmised due to the steric hindrance, which was found to decrease the formation of such complex with the [VO(tea)] and [VO(tpa)] analogs. Furthermore, the adducts formed in methanol and ethanol with [VO(tea)] were more stable with corresponding adducts formed with [VO(tpa)], and the fact that no adducts were observed for [VO(tpa)] in isopropanol and *tert*-butyl alcohol is consistent with the notion of steric hindrance in the more crowded complex.

This simple study illustrated the need to understand in detail the forms of V(V) in aqueous solution, although this description in this section is somewhat simplified because the solutions of vanadate at different pH levels also contains higher oligomeric vanadate species. However, these studies do highlight the fact that even a simple ligand such as tea has ample opportunities for complexes to form with vanadate and that of the possible four multidentate complexes that could form, and we found evidence that three did. This and other work documented the complexity of this chemistry, but importantly, an in depth understanding was achievable because the vanadium had the spectral probes needed to understand the system in detail.

4.2. Simple Buffers Including Good Buffers: Tricine. In the following, we describe the chemistry found with one good buffer with vanadate because it illustrates the kinetic properties in these complexes. Indeed, in introductory inorganic chemistry we learn that all coordination complexes are labile except those containing Cr(III), Co(III), and Ru(II). As a result, we anticipate that V(V) complexes would be labile. One of our early studies with one of these buffers, *N*-[tris(hydroxymethyl)-methylglycine], referred to as Tricine, investigated the formation of the V(V)−Tricine complex, Figure 8.[112] The reaction is shown in eq 3; however, the importance of the lability of these complexes and the consequence of the structural flexibility finds was not realized until recently.

$$H_2VO_4^- + H_2\text{Tricine} \rightleftharpoons [VO_2\text{Tricine}]^- + 2H_2O \quad (3)$$

The Tricine ligand is coordinated to the V(V) in a tridentate manner in the V−Tricine complex, and therefore, the complex has two free hydroxyl group ligands, which are different due to the assymmetry of the complex, see Figure 8.[112] As observed in the ^{13}C EXSY spectrum recorded at 273 K, the off-diagonal signals in the EXSY spectrum indicate that magnetization transfer moves from one position to the other;[24,105,112] that is, the spectrum in Figure 9a shows the C atom in the free

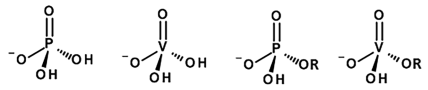

Figure 9. Illustration of the vanadate−phosphate analogy and the organic vanadate and organic phosphate analogy. Illustration of the structures of $H_2PO_4^-$, $H_2VO_4^-$, $H(CH_3)VO_4^-$, and $H(CH_3)$-PO_4^-.[9,21,63]

hydroxyl group connected with the C atom in the hydroxyl group in the V−Tricine complex. The ligand−V−Tricine complex exchange is indicated by solid lines in the EXSY spectrum connecting the off-diagonal signals between the V−Tricine complex and free Tricine C atoms. Interestingly, this intramolecular exchange reaction is only slightly faster than the

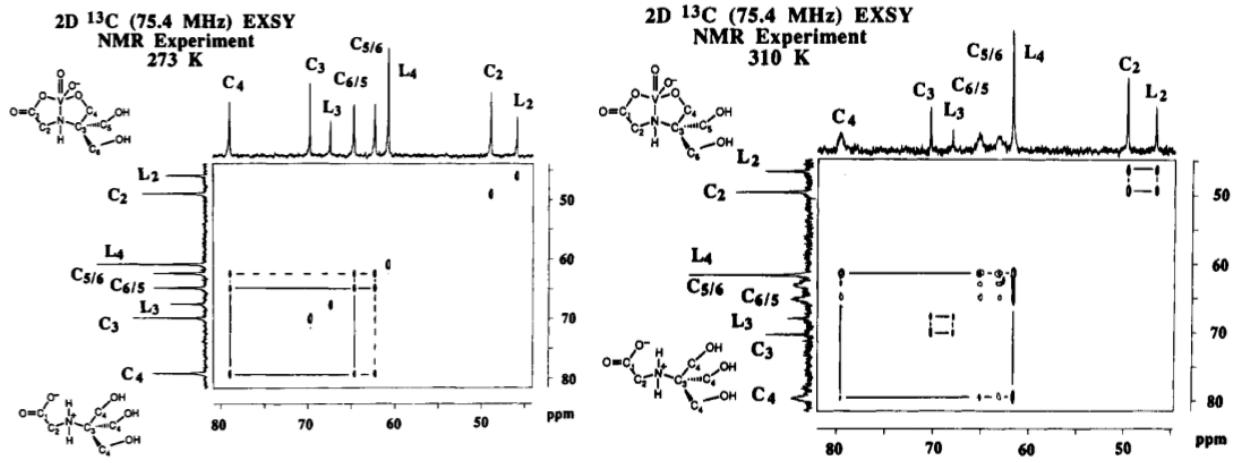

Figure 8. ^{13}C EXSY spectrum of 412 mM vanadate and 500 mM Tricine at pH 7.1 at 273 K containing 406 mM V-Tricine and 0.38 mM vanadate monomer (left) and of 412 mM vanadate and 500 mM Tricine at pH 7.2 at 310 K containing 398 mM V-Tricine and 1.2 mM vanadate monomer (right).[112]

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complex formation observed in the ^{13}C EXSY spectrum recorded at 310 K shown in Figure 8b.[112] As a matter of fact, some additional off-diagonal signals in the EXSY spectrum show the additional intramolecular process recorded at 273 K is also observed at this higher temperature.[112]

Structural studies were carried out that characterized both V complexes in solution and solid state.[24,103−106,111] Because the vanadium is a small early first-row transition-metal ion, it has a wide range of coordination chemistry preferences open to it and will form different systems depending on the specific conditions.[9] Some differences were observed when the environment becomes more hydrophobic indicative of changes in the stability of charged coordination structures in aqueous environment.[8] The possibility for formation of neutral structures in media with lower dielectric constants can also be very important because such species can partition up into interfaces as well.[113] Indeed, the changes in coordination geometries with environment showed early on the flexibility of the vanadium systems to adapt to the environment and carry out chemistry more aligned with the dielectric constant of the medium at hand.[111] We show that V(V) forms particularly strong complexes with the Tricine ligand and that this complex contains one carboxylate group.[105,106,112] Because of this, it is structurally different than the simpler $[VO_2(Htea)]^-$ complex.[103−106,111] When comparing the rate of V−Tricine formation with previous complexes, it is clear that that this complex as others follow a dissociative reaction pathway.[24,105,112] However, as we found for the V−Tricine complex, both intramolecular and intermolecular processes can take place under mild conditions and the exchange of the hydroxyl group is faster than ligand exchange.[112] This system demonstrates that multidentate ligands can engage in complex processes involving formation and conversion of vanadium complexes akin to those anticipated to be observed in V−protein complexes observed several decaded later.

4.3. Aqueous Vanadate Speciation and Their Conversions. Vanadium(V) is structurally, electronically, and, with regard to biological properties, a phosphate analogue Figure 9a,b.[9,21−23,69,114−116] However, vanadate in contrast to phosphate can undergo a multitude of reactions with simple buffers, metabolites, and even with itself. These reactions include protonation−deprotonation reactions and oligomerization reactions; see eqs 1−4 as representative examples. The pK_a values for monomeric vanadate, usually referred to by the generic term vanadate, are 3.5, 7.8, and 12.5, which compare to the corresponding pK_a values for the phosphate at 2.1, 7.2, and 12.7.[9,21,23] The main species at pH 7 is therefore $H_2VO_4^-$. Because the speciation of vanadate is so pH dependent, concentration, ionic strength, and temperature are important conditions that define the activity of the vanadium species that exist in the system at hand. Therefore, under most reactions in the laboratory, the concentrations are higher than they would be under biological conditions and oligomers will be present. Therefore, upon administration of a compound, the overall vanadium concentration is much lower, and thus, the concentrations of oligomers are much lower.[9,21,63] Therefore, unless the conditions change dramatically, the speciation of the vanadium would be anticipated to be monomeric although the pH would determine the charge of the oxovanadium species and whether it is $H_2VO_4^-$ or VO_2^+.

$$H_2VO_4^- + H_2O \rightleftharpoons HVO_4^{2-} + H_3O^+ \quad (4)$$

$$VO_2^+ + 2HO^- \rightleftharpoons H_2VO_4^- \quad (5)$$

$$H_2VO_4^- + H_2VO_4^- \rightleftharpoons H_2V_2O_7^{2-} + H_2O \quad (6)$$

$$4H_2VO_4^- + H_2VO_4^- \rightleftharpoons V_4O_{12}^{4-} + 4H_2O \quad (7)$$

The speciation diagram can take on many forms depending on whether the protonation reactions or the oligomerization reactions are of interest. Inside cells in the reducing environment of the cytoplasm, the vanadium(V) will reduce to form vanadium(IV), and in this case the speciation diagram can be illustrated as a Pourbaix diagram, as shown in Figure 10.[21] In most of the speciation diagrams, the species are defined

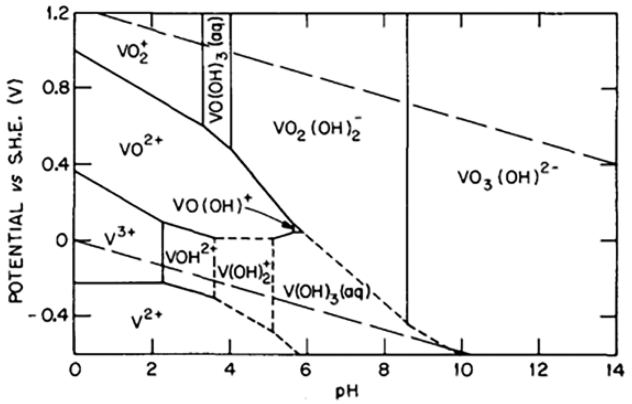

Figure 10. Pourbaix speciation diagram for vanadium. Species are shown at varying redox potential and as a function of pH. Adapted with permission from ref 21. 1983 Springer.

by composition, that is, stoichiometry. This characterization provides nuclearity and charge on the species, but since the number of water atoms are not defined, the coordination geometry is less specific. Indeed, the question of the coordination number of the vanadium in $H_2VO_4^-$ is somewhat unclear, and even recent studies are favoring a coordination number greater than 4, presumably forming species such as $VO(OH)_4^-$,[117] which is a species that was suggested several decades ago. Regardless of the exact coordination geometry of this species, the vanadate−phosphate analogy in biology is firmly established.[9,21,63]

The rapid interconversion that takes place in aqueous solutions is very important. These interconversions were established in several ways; however, the use of EXSY spectroscopy elegantly demonstrated that the oxovanadates all interconvert.[118,119] The observations of interconversions are very important because before these studies it was assumed that most of the observed biological responses were taking place in response to vanadate monomer.[120] With these studies we could verify that responses to, for example, one of the other oligomers are indeed possible because the enzyme responds faster than the millisecond time scale on which the exchange is occurring.[120−122]

4.4. Aqueous Vanadate Esters, Corresponding Oxovanadium Alkoxides, and Their Chemical and Biological Activities. The potential for organic vanadate esters to be inhibitors for phosphatases depends on their ability to form five-coordinate species and the stability of such species. This potential was explored in a range of studies. The ester-forming reaction of vanadate with simple alcohols in aqueous solution was initially described by Gresser and Tracey, and two seminal

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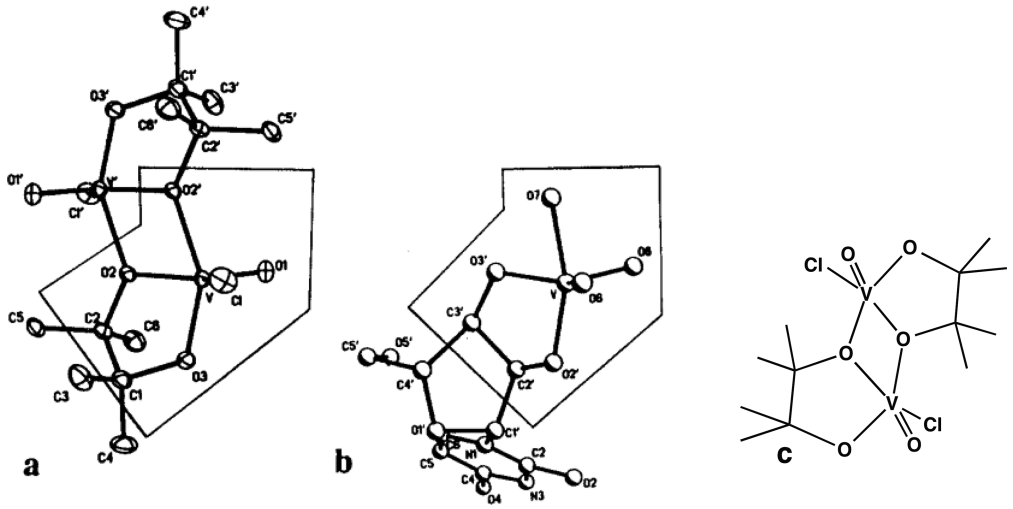

Figure 11. Comparison between the vanadium coordination geometry in the pinacol−vanadate complex (a) and in the uridine−vanadate complex inside the ribonuclease (b). Also included is the schematic illustration of the synthetic analogue (c).[126]

papers were reported by these investigators documenting the formation of the vanadate ester at ambient temperature for ethyl vanadate[123] followed by application of this reaction using a more complex substrate.[124] The second manuscript demonstrated the more complex substrates such as glucose and vanadate, and in this work, because so many products form, the enzyme glucose-6-phosphate dehydrogenase was used to demonstrate that the glucose-6-vanadate ester forms.[124] This system was explored later in further detail as described below.[111,125] Following this work, a series of elegant papers were reported exploring the variations in the stability of the vanadate esters and documenting the versatility of this reaction.

We became involved in the studies of organic vanadates from several perspectives, exploration of new systems, characterization of the structure of vanadate esters,[126−130] the kinetics of these compounds,[119] and their biological properties.[22,111,125] In one of the first reported studies involving the vanadate ester formation, we documented the substrate specificity of two aldolases and formation of less common carbohydrates. Specifically, fuculose-1-phosphate aldolase and rhamulose-1-phosphate aldolase catalyze a condensation reaction with dihydroxyacetone-1-vanadate.[22] Although the reaction documented the fact that these species are accepted as substrate analogues, the vanadate and/or the vanadate ester reduce under the reaction conditions and make the system much less practical and convenient to use in synthetic organic chemistry.

The organic vanadate esters were presumed to be excellent analogues of the organic phosphate esters; however, no structural evidence was reported until 1991 when the first vanadium alkoxide to pinacol was reported Figure 11.[126] The structure was presented as the first transition-state analogue for the vanadium−nucleoside complexes, and the illustration compared the geometry of the model compound with that of the vanadate−uridine complex inside ribonuclease. We have since then pointed out that the geometry of this compound, as in most five-coordinate small molecules, is distorted, and the geometry is closer to square pyramidal than trigonal bipyramidal (see below, Figure 14).[7,77] The coordination geometry at the vanadium atom is restricted by the diamond core V−O−V−O four-membered ring, as evidenced by the V−O bond length in the diamond core ring of 1.964(7)Å, the oxo group length at 1.576 Å, and the external V−O bond of 1.773 Å.[126] The subtle structural changes in the ligand are very dramatic for these systems because changes in the 1,2-diol from pinacol to ethylene glycol result in the diamond core four-membered ring opening, with bond lengths of 1.741(3)Å for the V−O and 1.578 Å for the V═O.[126] The distance between the V and the O atom in the open ring is about 3.7 Å compared to the 1.964(7) Å in the pinacol complex.[126] Additional structures were subsequently reported including a vanadium−2-hydroxybutyric acid complex[131] and a vanadium−adenosine complex.[132] These structures do document the increased length of the V−O bonds in these organic vanadates. The structural data suggests that the organic vanadate−organic phosphate analogy is limited to enzyme systems with more flexible active sites.

After the report on the pinacolate structure, other examples of vanadium alkoxide structures emerged, some with sterically hindered ligands[125] and some with ligands with some alkoxide functionalities[132,133] and other functionalities such as acids.[131] Because these systems also were of interest for catalytic purposes, a number of groups were working on such systems, which include simple alkoxides,[125] $VO(acac)_2$-derived molecules that oxidized during the preparation included both simple systems, as well as complex polyoxometalates.[133,134] Unfortunately, these systems are not always characterizable by X-ray crystallography. In cases when the compounds resist characterization, we used solid-state ^{51}V and ^{13}C NMR spectroscopy to elucidate the structure of the materials formed.[129] Although the focus of my group has generally been on vanadium(V) systems, some vanadium(IV) complexes have also resulted.[56] Combined with work carried out by other groups on related systems, the structure and reactivity of these systems are much better understood. With the assistance of theoretical methods, the reactivity and structure of these compounds have been further investigated.[115,135]

In studies probing the nature of the vanadate ester complexes, investigations carried out in the presence of buffers[66] led to demonstration that the kinetics of the organic vanadate ester formation[119] changed in the presence of an imidazole buffers.[119] In addition, in solutions of nucleoside (adenosine and uridine), vanadate and imidazole in addition to the 1:1 vanadate−nucleoside and the 2:2 vanadate−nucleoside complexes formed a new complex with a stoichiometry 1:1:1 of

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vanadate−nucleoside−imidazole.[109,136] This complex was very stable, demonstrating the stabilizing effects that a ternary ligand can have on the vanadate−nucleoside complex. The interest in these complexes was initially high because ribonuclease was found to bind a vanadate−uridine complex.[109] A crystal structure of vanadate−uridine nucleoside bound to ribonuclease was reported; however, the structure was not deposited in the Protein Database because there was not 100% occupancy at the protein active site. The potential that the 1:1 complexes were AMP (AMV) and UMP (UMV) analogues was intriguing, but the major complex that formed in these systems was the 2:2 vanadate−nucleoside complexes.[136] Several structural proposals were made to describe this complex with isotopically enriched ^{17}O NMR studies leading to the final proposal with a diamond core V−O−V−O four-membered ring supported by the 1,2-diol.[130] This structure was soon thereafter supported by the crystal structure of a 2:2 vanadate−adenosine complex.[132]

The potential greater impact of making a vanadate ester forming a cofactor such an ester would have wider application, and we explored the possibility of making the vanadate analogue of NADP.[116,137] Since NAD contains two 1,2-diol moieties, the resulting compound adducts are likely to have at least one major 2:2 complex. However, when vanadate is added to a solution of glucose-6-phosphate, NAD, and glucose-6-phosphate dehydrogenase from bakers' yeast the rate of glucose-6-phosphate oxidation is dramatically increased.[125] Similar studies were carried out with alcohol dehydrogenase (ADH) from *T. brockii*, and in Figure 12 we show the difference

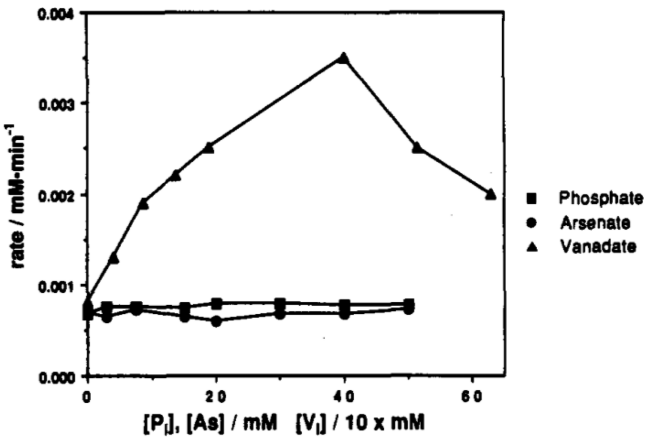

Figure 12. Rate of ADH-catalyzed oxidation of ethanol as a function of phosphate, arsenate and vanadate concentration.[111]

in rate by addition of phosphate, arsenate, and vanadate.[111] The concentrations of phosphate and arsenate are up to 10-fold higher than vanadate and still did not show comparable oxidation rates. Analysis was carried out, and it was found that the oxidation rate was proportional only to the concentration of vanadate monomer. These results showed that the presumed NADV (2′-NADV) is a better cofactor analogue for ADH than both NADP (2′-NADP) and c-2′,3′-NADP by a factor of 100 as defined by k_{cat}/K_m.[111] These differences in the substrate specificity show that there are some distinct differences, presumably in the active site of the enzymes leading to a more favorable processing. Indeed, the ADH from *T. brockii* has a higher affinity for both NADP and c-2′,3′-NADP cofactor analogues compared to glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenases from bakers' yeast and sheep liver; however, together these studies show that an organic vanadate ester can effectively serve as a NADP cofactor analog.[111]

In the early 2000s, we were involved in investigating the insulin-enhancing effects of a range of vanadium compounds including complexes with all-O atom coordination spheres.[56−58,138] In addition to salt complexes, complexes such as $VO(acac)_2$ and derivatives,[57,138] a peroxovanadium complex,[139] and BMOV contain only all-oxygen atoms in their coordination sphere.[15,71,140−142] $VO(acac)_2$ is a oxidant and a compound often used for oxidation reactions;[143−145] however, in a collaborative study with Brichard, three derivatives were investigated as antidiabetic agents ($VO(acac)_2$, $VO(acac\text{-}Me)_2$, and $VO(acac\text{-}Et)_2$).[56,57] $VO(acac)_2$ corrected the hyperglycemia and impaired hepatic glycolysis of diabetic rats more safely and potently than vanadyl sulfate ($VOSO_4$) and the other two $VO(acac)_2$ derivatives. This is not simply due to improved intestinal absorption but also indicates more potent insulin-like properties. The peroxovanadium compound was found to have effective in vitro antidiabetic effects, and its structure remains one of the few X-ray structures of imidazole coordinated to vanadium.[139] Finally, recently the effects of BMOV and $[VO(malto)_2]^-$ on the cellular membrane and model membrane systems were explored.[10,15,140−142] The compounds were not found to interact with the insulin receptor (IR), and the IR surface density was not effected either. However, BMOV was found to interact directly with the plasma membrane, driving changes in lipid order and leading to increased compartmentalization of IR and insulin receptor substrate-1.[15]

In the past decade, we have been exploring the properties of vanadium complexes in the presence of interfaces and how their reactivity changes in such a heterogeneous environment. In an early study, we demonstrated that the ligand is very important for how the vanadium is processed and taken up by cells.[10,11] Specifically, human erythrocytes treated with the DIDS inhibitor were not able to take up vanadate; however, $VO(acac)_2$ and BMOV were readily taken up.[10] This finding was consistent with two studies documenting that complexes with the same ligand but different oxidation state of the vanadium yielded different responses.[35,49,50] These studies have led us to investigate the interaction with a variety of model membrane systems[8,15,71,146−149] and the effects of different vanadium compounds on membrane associated proteins.[15,71] For example, in a study with Ca^{2+}-ATPases we demonstrated a significant difference between amavadine, BMOV, and $[VO_2(dipic)]^-$ with the latter being the most potent inhibitor.[71] A similar pattern was observed for the inhibitory affinity of this series of compounds as inhibitors for PTP1B.[72]

Combined, these studies demonstrated that the vanadium can form four- and five-coordinate organic vanadates and that they can act as substrates and inhibitors for enzymes. The specific mode of action therefore depends on the enzyme system and in some cases the environment. These studies illustrate a flexibility of the organic vanadate esters and that should an environment exist in which the complex is stabilized the complex undoubtedly will form.

4.5. Interactions of Vanadate with Other Metabolites.

Vanadate interacts with numerous other cellular components ranging from simple anions such as chloride to more complex systems such as amino acids, peptides, and proteins such as transferrin. In addition to proteins, the metabolites which are probably the most important from a cellular perspective are phosphate and phosphate derivatives.[23,109,132,136,150−153] The

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reactions between vanadate and phosphate have been characterized in detail by Tracey and co-workers.[150,151] Vanadate can form analogues of ATP and in some systems will be recognized as such. Other important metabolites that vanadate interacts with include ascorbate and gluthathione. The reaction of vanadate with ascorbate has also been reinvestigated, and both inner and outer electron-transfer processes were detected.[113,154,155] These are important because they maintain the cellular redox potential. Some controversy existed with regard to whether vanadate reduced in the presence of and upon reaction with thiols, and we demonstrated that the formation of complexes could change the redox potential and that pH and concentration are also important for whether the vanadium reduced or not.[79] Redox chemistry facilitated by vanadium is a very important area considering the relationship with oxidative stress and formation of ROS.[6] These processes relate to the cellular redox cycling and are undoubtedly important for how the vanadium compounds act.

Vanadium binds tightly to many proteins in cells including transferrin.[64,80−82] Vanadium is likely to be bound to transferrin before it gets to the cell because transferrin provides an avenue for the vanadium to be transported in the blood. However, how the vanadium would dissociate from the transferrin upon reaching the active site and active pool is less obvious.[4,5,64,80] Indeed, binding of the vanadium to the transferrin may be in part responsible for why the doses of vanadium are as high as they are in order to achieve anti-diabetic phenotypes.

5. V−PROTEIN COMPLEXES CHARACTERIZED BY X-RAY CRYSTALLOGRAPHY

In 1985, the first V−phosphorylase complex, namely an uridine−vanadate−ribonuclease A complex, was reported,[83] although the first V−protein phosphatase complexes were only reported much later (the first PTP1B X-ray structure was reported in 1994−1995).[92] In 1997, the first V−protein phosphatase complex was characterized,[156] and in 1999, the first V−alkaline phosphatase complex was characterized.[157] In 2014, the vanadium−phosphatase complexes had increased to about 40, and in a review it was documented how useful vanadium is for crystallization of phosphatases and other phosphorylases, haloperoxidases, bromoperoxidases, and chloroperoxidases.[77,83] Currently, we have about 100 vanadium−protein complexes as recently described in several reviews.[25,77,83] At this point, there is a significant body of data that will allow us to examine the preferences of the proteins for the vanadium (Figure 13).

6. X-RAY CRYSTALLOGRAPHY OF V−PHOSPHATASE COMPLEXES WITH VO_4O COORDINATION GEOMETRY

The vanadium-facilitated inhibition of phosphatases is believed to involve forming five-coordinate transition state−protein complexes.[6,9,21,59,63,65,72,77,159,160] The two most common coordination geometries for five-coordinate vanadium are square pyramidal and trigonal bipyramidal. As shown in Figure 14, the Addison rule determines tau (τ) and allows readily the distinction of those two geometries.[158] The square-pyramidal geometry has a τ value of 0 and the trigonal bipyramidal geometry has a τ value of 1. Although a five-coordinate geometry is expected for the active site enzymes going by an associate mechanism, the coordination geometry may vary somewhat for the different phosphatases. Because a number of

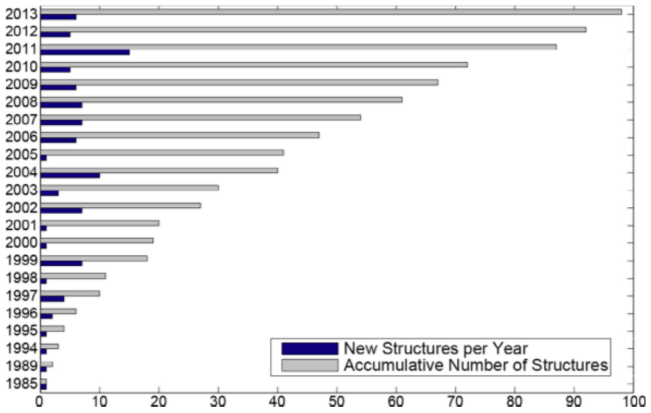

Figure 13. Publication of V−protein complexes in the PDB. The black bars represent annual new structures and gray bar represent total structures. Adapted with permission from ref 83. 2014 Elsevier.

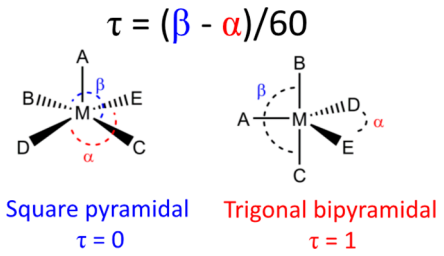

Figure 14. Square pyramidal and trigonal bipyramidal geometries are shown. Using the calculation for the τ value the square pyramidal is 0 and the trigonal bipyramidal is 1.[25,77,158]

these vanadium−phosphatase complexes, which form during the hydrolysis of organic phosphate esters, have been isolated and structurally characterized, we can examine these complexes. Moreover, phosphatases, which hydrolyze organic esters through a concerted mechanism, are also known to form vanadium−phosphatase complexes.[60,84] Because the coordination geometry of the vanadium in the active site of these enzymes is not known, we initiated a data-mining project to investigate this question. Specifically, we examined the X-ray structures of vanadium−phosphatase complexes with the objective of determining the coordination chemistry of the vanadium in the known vanadium−proteins complexes of all phosphatases regardless of their mechanism of the reaction.[7,77]

The X-ray structures of vanadium−phosphatase complexes deposited in the protein data bank were downloaded. The data mining for the 44 vanadium−phosphatase complexes was carried out by peeling off the peptide from the protein until the active site is located. At that point, the two largest angles were identified, and the τ value was calculated. There are 14 V phosphatase complexes containing VO_4O in the active site. The details of the actives sites were reported previously and summarized in Table 1 for the alkaline phosphatases and for PTP1B.[77]

The parameters listed in Table 1 for the phosphatases containing an O-residue in the active site show that most of the vanadium−phosphatase complexes have the VO_4 bound to an O-residue in a TBP geometry in the active site. Of the 14 structures, 12 have a τ value above 0.5 and two are below 0.5. One of these two structures with τ value below 0.5 (Refcode 1J9L) contains a OSer residue at a distance of 3.617 Å from the vanadium.[163] That is a very long distance and outside the range of a bond. Thus, this V atom is not properly characterized as a

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Table 1. Known Phosphatase Structures with Ser and Thr in the Active Site Containing Vanadium and Some Details about Them as Well as PTP1B[77]

PBD ID no.	source	enzyme	EC no.[a]	form of V in active site	τ value	res (Å)	R_{Obs}	geometry[a]	exposure to V	pH	ref
3e81	*Bacteroides thetaiotaomicron*	bact KDN-9P	NR	VO3··· Asp.cofactor	0.95	1.63	0.176	TBP	soak	8.50	161
1b8j	*Escherichia coli*	alkaline phosphatase	3.1.3.1	VO4···Ser	0.77, 0.85	1.90	0.171	TBP	soak	7.50	157
3t00	*Sinorhizobium melioti*	phosphoacetate hydrolase	3.6.1.9	VO4···Thr	0.83	1.80	0.207	TBP	soak	7.50	162
1j9l	*Thermotoga maritima*	Survival protein E (SurE)	3.1.3.5	VO4···H bonds	0.43	1.90	0.199	distorted tetrahedral	soak	7.50	163
2gso	*Xanthomonus ananopodis*	NPP	3.6.1.9	VO4···Thr	0.55	1.75	0.173	TBP	CO	6.00	164
2d1g	*Francisella tularensis*	acid phosphatase A (AcpA)	3.1.3.2	HVO_4^{2-}	0.76	1.75	0.198	TBP	soak	6.00	165
2rbk	*B. thetaiotaomicron*	HPP wild type		O···VO3···Asp	0.78	1.00	0.123	TBP	soak	5.50	61
2rar	*B. thetaiotaomicron*	HPP D10A mutant		VO3···Asp	tetrahedral	1.52	0.167	tetrahedral	CO	7.00	61
3zwk	*Thermus thermophilus*	mannosyl-3-phosphoglycerate	3.1.3.70	VO3···W···Asp	0.56	2.10	0.158	TBP	soak	6.50	166
3zx55	*T. thermophilus*	mannosyl-3-phosphoglycerate	3.1.3.70	VO3···Asp···MG	0.89	1.81	0.170	TBP	soak	6.50	166
4knw	*Homo sapiens*	*N*-acylneuraminate-9-phosphatase	3.1.3.29	VO4···Asp	1.07	2.70	0.255	TBP	soak	7.50	167
3zwu	*Pseudomonas fluoroscens*	alkaline phosphatase Phos	3.1.3.1	VO5	0.94	1.39	0.159	TBP	NR	8.00	168
4kkz	*Phaseolus vulgaris*	Purple acid phosphatase	3.1.3.2	VO4···OEt	0.83	2.20	0.153	TBP	NR	4.0	169
3f9b	*Yersinia enterocolitica* (type O:9)	W354F PTPase	3.1.3.48	V2O7(VO4···Cys; VO5)	0.07/0.78	1.42	0.174	SP/TBP	CO	7.50	52
3i80[b]	*H. sapiens*	PTP1B[b]	3.1.3.48	VO4···Cys	0.90	2.25	0.201	TBP	CO	7.50	60
3i7z[b]	*H. sapiens*	PTP1B[b]	3.1.3.48	VO4···Cys··· DADEYL peptide	0.83	2.30	0.207	TBP	CO	7.50	60

[a]EC no.: enzyme categorization scheme. NR: not relevant. TBP: trigonal bipyramidal. CO: cocrystallized. [b]The active-site residue is a cysteine.[52]

five-coordinate V−phosphatase complex.[163] The second Refcode 3F9B with the VO5 geometry and a τ value for the V atom below 0.5 is part of a vanadate dimer that is coordinated in the active site.[52] The free V atom, which is not coordinated to the protein, does not appear to be subjected to the similar effects and therefore remains square pyramidal as preferred for small molecules (see below for further detail). The second V atom in the structure of the Refcode 3F9B is coordinated to the S atom of the cysteine in the PTP1B protein and is TBP.[156] Therefore, two outliers are not members of the family of V centers in proteins with coordination environments VO_4O directly coordinated to the protein. We conclude that the coordination chemistry of the V−O-active site containing phosphatases all have τ values above 0.5. The phosphatases with N-nucleophile and S-nucleophiles in the active site were also investigated with similar results.[77]

To be able to compare the vanadium in the protein structures, we needed to analyze all 3600 vanadium structures in small molecules.[7,77] The search showed that the vanadium complexes in small molecules were strongly favor the square pyramidal geometry. Of the 2710 molecules investigated in the case of the VO_4O geometry, the square-pyramidal geometry was favored by 97% and only 3% was TBP. Similar results were obtained, albeit not as dramatic, with both the V−phosphatase protein complexes with VO_4N and VO_4S coordination spheres in the active site. For the 368 compounds with the VO_4N geometry, the square-pyramidal geometry was favored by 75% and 25% was TBP.[7] For the VO_4S geometry there is only one structure, which is why we included moieties other than S in this category. For the final 44 molecules with the VO_4X geometry everything except for O and N was included for X. This category mainly consists of halogens, but also a C atom and a Cr-atom are represented.[7] Despite this structural variability, the square-pyramidal geometry was favored by 66% with only 34% TBP.[7] The data-mining studies for the small molecules clearly demonstrate that the main small molecule geometry for the vanadium atom is square pyramidal.

7. X-RAY CRYSTALLOGRAPHY OF V−PTP1B COMPLEXES WITH VO_4S COORDINATION GEOMETRY

Most of the 44 V−phosphatase complexes are simply vanadate bound to the protein. Of particular interest to the topic of this work are the V−protein complexes in which the vanadium is not simply a vanadate molecule. Considering specifically the PTP1B phosphatase, such examples are particularly relevant to the signal transduction processes and the antidiabetic effects of vanadium compounds. Therefore, we will turn the analysis at this point to the PTP1B X-ray complexes that we also listed in Table 1.[60] As shown in Figure 5, two energy maxima form between vanadate and PTP1B, including a TSA1 complex and a TSA2 complex.[60]

The PTP1B phosphatase has been studied in detail from several sources, and much is known about this enzyme with regard to structure, function, and mechanism.[25,73,78,87,98,170−172] Of particular interest to this work are the V complexes that have been described with the *H. sapiens* enzyme.[60] First, it is important to point out that the PTP1B is a phosphatase that is known to undergo hydrolysis through a conserted mechanism.[60,84] Thus, the observed inhibition by vanadate through a

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five-coordinate transition state structure was not expected as illustrated in Figure 15 using the ball-and-stick model.[60]

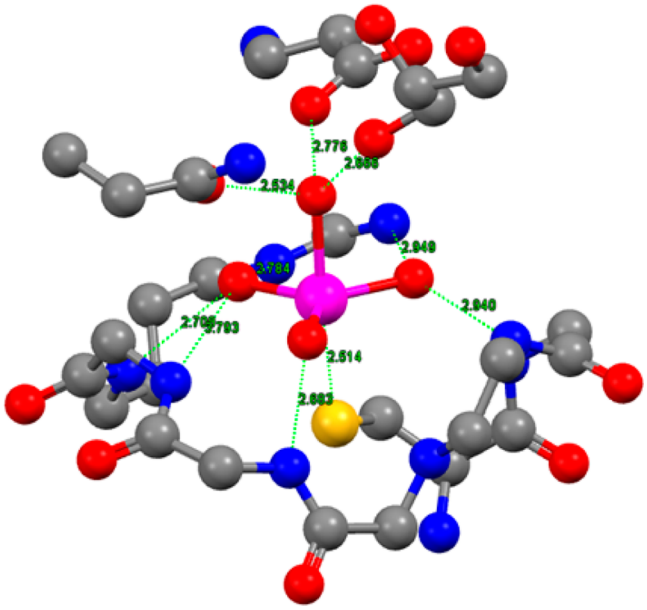

Figure 15. TSA2 complex using ball and stick models highlighting the H-bond stabilization with the PTP1B from *H. sapiens*.

However, the five-coordinate complexes have been structurally demonstrated as shown in Figure 6 and Table 1 in this work. We propose that the V−PTP1B complex forms because the large size of the vanadium in vanadate supports the exploded transition states both in TSA1 and in TSA2 protein-structures.[92] The substrate peptide was reported to bind exceedingly tightly,[60] and a similarly tight complex can be expected for the vanadate peptide complex, which has been characterized by X-ray crystallography.[60] The active site of PTP1B binds the vanadate and DADEYL peptide very tightly as the active site is supporting two or more H-bonds to each O-atom in the vanadate coordinated in the active site. Each oxygen atom in the vanadate is H-bonded to two or more groups stabilize the complex. The V−phosphatase complexes with the peptide substrate bind very tightly in the active site; however, the substrate extends from the active site cavity to an adjacent cavities on the protein. This allows the peptide to form additional stabilizing interactions. In Figure 16, the peptide substrate is shown to H-bond further to the phosphatase with highlighting interactions from the binding groove next to the active site.[60]

It has been recognized for some time that supporting interactions between a ligand and an enzyme are an effective way to strengthen the affinity of an inhibitor.[173,174] However, the inherent stability of the five-coordinate TBP geometry of the vanadium over the square pyramidal in the protein environment suggests a change in the electronic properties of the complex, presumably the molecular orbital arrangement in the TBP geometry. Importantly, the structures that have been captured by X-ray crystallography show the geometry of the vanadium is distorted toward umbrella geometry. Considering that these are exploded transition states, the specific umbrella and detailed geometry of the transition state defines the location of the ligands in the near apical positions of the vanadium coordination geometry.

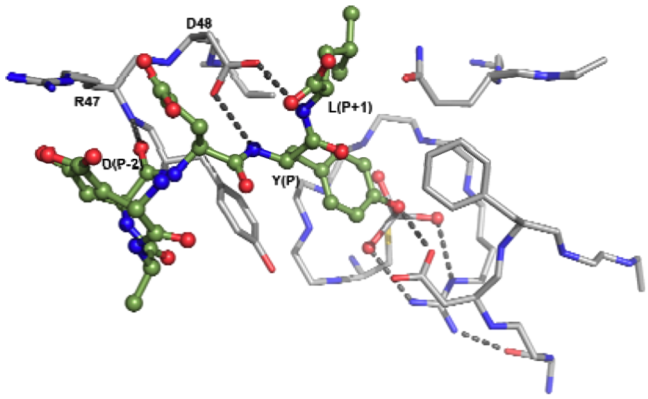

Figure 16. DADEYL peptide complex is forming H-bonds to the phosphatase along the peptide ligand and in this manner further strengthen the binding of substrate to the protein.[60]

Importantly, these observations are akin to those observations several decades ago where the chemistry of simple vanadium complexes was investigated. That is, the early report regarding the [VO(tea)] complex[111] which changed geometry with the polarity of the medium forming $[VO_2tea]^-$ in aqueous solution has set the stage for the subsequent observations described here. The ability of the organic vanadate ester to form both substrate and transition-state analogues has been demonstrated both in solution and in the solid state. Four-coordinate derivatives and five-coordinate derivatives form readily, and the specific details are very sensitive to the ligand and the environment of the compound. We have here shown that the properties for the small molecules are mirrored for the V−protein complexes. However, because many of the proteins involved undergo concerted reaction mechanisms, the five-coordinate transition-state analogue is probably better described as a high energy exploded geometry. Recently, reports of catalytic properties of related $[VOdipic]^-$ complexes extracted into organic solvent has led to application in lignin catalysis.[175−177] Similarly, the catalysis with [VO(tea)] and other derivatives have been reported.[145,178] The general observation that the properties of these molecules adapt to the environment in order to perform the task at hand is therefore important in a general sense.

8. DEVELOPMENT OF VANADIUM-CONTAINING PHOSPHATASE INHIBITORS AND ANTIDIABETIC COMPOUNDS IN THE FUTURE

Structural studies have been carried out characterizing V complexes in both solution and the solid state and defining their properties in detail. Because vanadium is a small early first-row transition-metal ion, it has a wide range of coordination geometries open to it and will form different systems depending on the specific conditions and environment. Indeed, anticipated changes based on polarities of the medium and the ligand may affect the resulting structure. A neutral compound will favor the higher coordination number and may support six-coordination at the vanadium center. In contrast polar ligands will engage in H-bonding of the vanadate and around the ligand. Indeed, structural changes with changing environment showed early on the flexibility of the vanadium systems to adapt to the environment and carry out chemistry more aligned with the dielectric constant of a particular medium at hand. As described in this paper, these properties were found to be critical for

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effects of vanadium compounds as phosphatase inhibitors and for antidiabetic studies.

A comparison of reported small molecule inhibitors with V−protein structures was carried out with the objective of developing guidelines for design and synthesis of more competitive phosphatase inhibitors.[7,77] In this comparison, we find, somewhat surprisingly, that all the reported small molecule vanadium-based phosphatase inhibitors are within a factor of 1000 in terms of inhibitor potency[179] and significantly less than the DADEYL−vanadate inhibitor bound in the X-ray structure of PTP1B.[60] These result were obtained considering compound stability and speciation.[63,101] We conclude that the inhibitory potency of the vanadium complexes can be enhanced dramatically by allowing additional interactions between the organic ligand and the phosphatase as in the case of the DADEYL peptide substrate. These considerations point to a frameshift in ligand design for vanadium complexes for development of potent inhibitors. Although such considerations have been appreciated among medicinal chemists,[60] the origin of the additional affinity gained by the exploded transition-state geometry stabilization of the vanadium trigonal bipyramidal geometry has not been appropriately appreciated.

The possibility that pharmacokinetics would support compound delivery to the PTP1B would require that small molecules would remain intact or reform upon administration. Studies using double-labeled complexes with a ^{14}C-containing ligand and ^{48}V-labeled V showed that the radioactivity from the ligand and the metal ion were not distributed similarly.[180] Indeed, studies have demonstrated that transferrin does bind vanadium tightly[37,62,80−82] and thus likely will impact the delivery and distribution of the vanadium.[63,64,153] Undoubtedly, ligands impact how the vanadium is taken up, and it is likely that the transferrin uptake of vanadium is affected by the ligands and their ability to shuttle the vanadium to the desired cells. Our studies have shown that there are large differences in how relatively similar vanadium complexes are processed. Furthermore, our recent studies in humans demonstrated that the concentration of V in serum did not correlate with clinical efficacy.[5] This implies that vanadium in serum or in any other compartment in equilibrium with serum V is not the active pool of vanadium. Since the active form of antidiabetic vanadium compounds still remains to be elucidated, and it is known that transferrin can bind and transport vanadium in blood, it is likely that the ligands of vanadium compounds are involved in shuttling the vanadium through membranes and to the active site.[37,62,80−82,181,182] As described in this work, potent inhibitors do exist, and should the delivery of these compounds intact to the target phosphatase become possible,[181,183] the concentrations needed for antidiabetic effects would dramatically decrease. If the concentration of the vanadium compound decreased the toxicity issues with these compounds would disappear. Future investigations into vanadium compounds should consider distribution of the vanadium and its delivery to the cells and the presumed active pool of vanadium. Combined, these studies do suggest that if the delivery of the vanadium and the peptidic substrate (or other similar ligand) could be done, the toxicity issues currently existing with the vanadate salts should be alleviated.

AUTHOR INFORMATION

Corresponding Author

*E-mail: debbie.crans@colostate.edu.

Notes

The authors declare no competing financial interest.

Biography

Debbie C. Crans is a Professor of Organic and Inorganic Chemistry and in the Chemical Biology and the Cell and Molecular Biology Programs at Colorado State University and is currently a Professor Laureate of the College of Natural Sciences. She did her undergraduate studies at the H. C. Ørsted Institute in Copenhagen, Denmark, in Biochemistry and Organic Chemistry. She received her M.S. and Ph.D. from Harvard University working with Professor George M. Whitesides. After postdoctoral studies at the University of California, Los Angeles, with Professors Orville L. Chapman and Paul D. Boyer, she joined the faculty at Colorado State University in 1987. She started the International Vanadium Symposium and Vanadium Society and the Zing Coordination Chemistry Conferences. She was Program Chair for the Inorganic Division for more than a decade and is the past chair for the Division of Inorganic Chemistry and the chair elect of the Colorado ACS section. She is a 2015 Author Cope Scholar, 2011 Colorado ACS Section Research Awardee, 2011 Best Teacher award, and a 2004 Vanadis Awardee. She is an ACS fellow and an AAAS fellow and has been a Senior Humboldt Awardee and a Japan Society of Promotion of Science Fellow. Dr. Crans has published over 180 original research papers in fundamental areas of inorganic and organic chemistry often applied to biological or medical problems. Her expertise includes metals in medicine, coordination chemistry, speciation, chemistry at the nanoscale, spectroscopy, and enzymology, and she studies diseases such as diabetes and tuberculosis. She continues to work in the areas of metals in medicine including vanadium-based drugs as antidiabetic or anticancer agents, amyloid metal chemistry, and nanoscale reactions in confined spaces. In addition, she is currently also involved in studies of the mode of action of pyrazinamide and other antituberculosis drugs.

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