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Review

How environment affects drug activity: Localization, compartmentalization and reactions of a vanadium insulin-enhancing compound, dipicolinatooxovanadium(V)

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ABSTRACT

The chemical and biological properties of a simple and traditional V(5+) coordination complex, dipicolinatooxovanadium(V) (abbreviated $[VO_2dipic]^-$), are described in order to present a hypothesis for a novel mode of action wherein a hydrophobic membrane environment plays a key role. Specifically, we propose that the compartmentalization and both chemical and biological transformations of vanadium-complexes direct whether beneficial or toxic effects will be observed with this class of compounds. This concept is based on the formation of high levels of uncontrollable reactive oxygen species (ROS) from one-electron reactions or alternative events possibly initiated by a two-electron reaction which may be directly or indirectly beneficial by reducing the high levels of ROS. The properties of dipicolinatooxovanadium(V) compounds in aqueous solution (D.C. Crans, et al., Inorg. Chem. 39 (2000) 4409–4416) are very different from those in organic solvents (S.K. Hanson, et al., J. Am. Chem. Soc. 131 (2009) 428–429) and these differences may be key for their mode of action. Since other vanadium complexes are known to hydrolyze upon administration, the low stability of the aqueous complex requires entrapment in hydrophobic environments for such a complex to exist sufficiently long to have an effect. The suggestion that the environment changes the reactivity of the compounds is consistent with the very different modes of action by which one complex act. In short, a novel hypothesis is presented for a mode of action of vanadium compounds based on differences in properties resulting from environmental conditions. These considerations are supported by recent evidence supporting a role for membranes and signal transduction events (D.A. Roess, et al. Chem. Biodivers. 5 (2008) 1558–1570) of the insulin-enhancing properties of these compounds.

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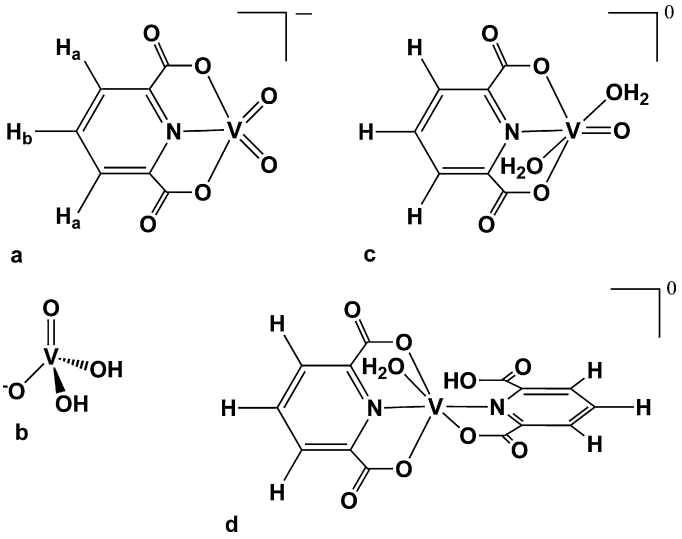
1. The action of vanadium compounds can be beneficial and malicious; could chemical and biological transformation be related to function?

Design, efficacy, and mode of action are defining properties of all drugs, including metal-containing drugs [1,2]. Drug efficacy is dependent on absorption, distribution, metabolism and excretion, properties that are disease- and organism-specific, and ultimately dictate mechanism of action. The action of a drug generally describes how the product impacts cellular function resulting in the reduction of a disease state. Drug design is often linked to its function and cellular uptake mechanism to facilitate efficacy. Pharmacokinetic studies characterize distribution and transformations that occur during drug function. Since the success of a drug is often a balance between beneficial and toxic concentrations, the greater the difference between therapeutic and toxic levels, the better. Targeting improves the benefit of a drug due to the inherent reduction in risk of toxicities in a host (i.e. by lowering potential systemic levels and the attainment of toxic levels) while still achieving therapeutic results. Therefore, for the successful development and administration of any drug, detailed information on its transformation and localization *in situ* is critical. Herein, we review available evidence regarding the biotransformation [3–9], localization [4,5,8,10–15], and toxicity [8,16,17] of one vanadium(V)-containing insulin-enhancing agent, 2,6-pyridinedicarboxylato-oxovanadium(V) ($[VO_2dipic]^-$; Scheme 1) [3–7,9,12,18–23], reconciling its beneficial and malevolent effects in cells and in diabetic animals with its chemical properties.

Vanadium (abbreviated as V) compounds, where salts are considered charged complexes, are particularly sensitive to their environment [24–27]. Importantly, various forms of V exert different biological activities [24,28–31]. It is well known that V salts and compounds undergo biotransformations (summarized in Fig. 1). Undoubtedly, the degree to which pentavalent V(5+) is reduced to tetravalent V(4+) is an important factor influencing how much metal/agent is transported into/out of cells, the magnitude of detoxification reactions initiated, how extensively superoxide



Scheme 1. The structures of the (a) oxovanadium(V) dipicolinate ($[VO_2dipic]^-$) (b) vanadate, the V(5+) salt diprotonated anion, $H_2VO_4^-$ (c) oxovanadium(IV) dipicolinate $[VOdipic(H_2O)_2]$ (d) hydrogen bis(dipicolinato)vanadium(III) $H[V(dipic)_2H_2O]$.

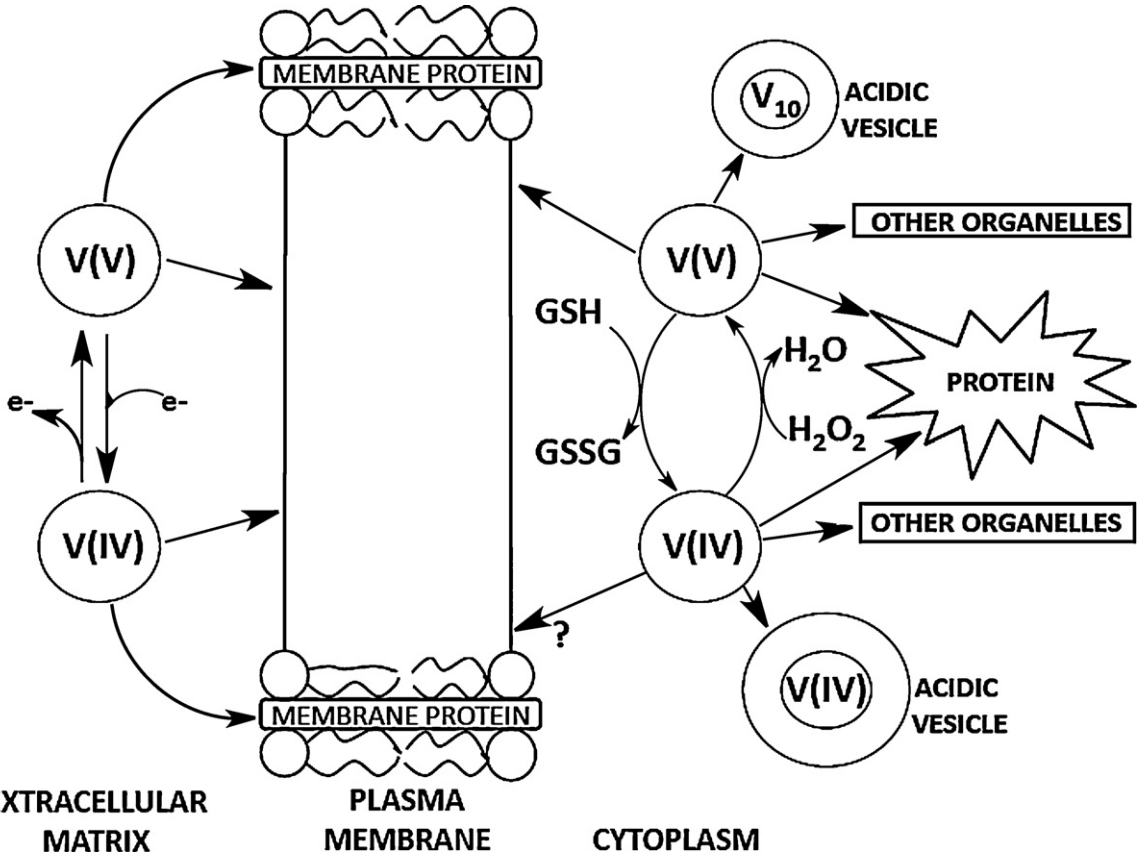


Fig. 1. An illustration of the current reported vanadium interactions in biological systems, with one electron V(4+/5+) redox chemistry occuring in the extracellular matrix (ECM). Membrane interactions are shown occuring via either passive diffusion of membrane protein interaction mechanisms. Upon entry into the cytoplasm V(4+) and (5+) affect cellular components in many ways.

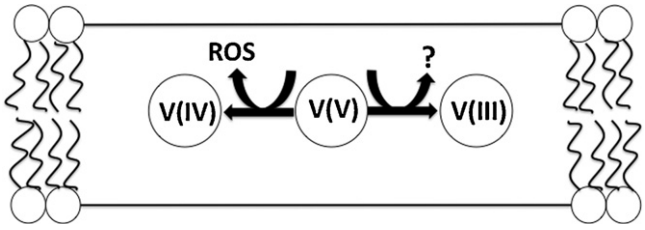


Fig. 2. Vanadium(3+), (4+) and (5+) salts and complexes enter by passive diffusion through the hydrophobic intermembrane space. The hydrophobic environment results in altered reactivity of the V complex. The formation of excessive amounts of reactive oxygen species (ROS) from V(5+/4+) redox results in toxic responses. Alternatively, the more controlled V(5+/3+) redox pathway result in unknown beneficial products.

anion ($^{\bullet}O_2^-$) and hydrogen peroxide (H_2O_2) metabolism is affected (also referred to as reactive oxygen species formation), as well as the extent to which several key cellular processes are potentially impacted [14,16,32]. In this review, we evaluate the hypothesis that some novel chemistry that has recently been reported in organic solvents using catalytic V compounds [33–38] could apply to hydrophobic environments of the cell and as such, be relevant for the mode of action of vanadium compounds. Hydrophobic environments exist in the intermembrane spaces of bilayers and in various locations within folded proteins that are found throughout a cell. Fig. 2 illustrates two very different chemical events occurring in a hydrophobic membrane environment based on known chemistry in organic solvents. The one-electron process is accompanied by reactive oxygen species (ROS) and reactive oxygen nitrogen species (RONS) formation. The two-electron process, although much more rare, recent reports with the V-dipic system in organic solution suggest that this process may be possible [33–35]. An important consequence of this hypothesis is that the effects of V-based drugs may change depending on environment. Thus, more so than with carbon-based drugs, the location and compartmentalization of the V-containing drug are factors critical to the actions of these agents.

Vanadium compounds exert a range of biologic/pharmacological effects. Both V(4+) and V(5+) compounds result in insulin-enhancing responses in both diabetic humans and animal models [22,24,39–44]. The insulin-enhancing effect of one particular V(5+) compound, $[VO_2dipic]^-$, is shown and compared to its corresponding free ligand and simple salt in Fig. 3 [3] and shows that the complex induces a statistically different response on Wistar rats with STZ-induced diabetes. This manuscript briefly reviews the insulin-enhancing effects of V-dipic compounds, [3,4,7–9,19,20,22] their effects reported on the cell membranes, [8,12,14,15] and aims to reconcile these effects with recently reported catalytic chemistry taking place in hydrophobic environments [33–35]. Based on known chemistry, we explain the biological activities of these compounds in the context of their fundamental physical and chemical properties in a hydrophobic membrane environment. Accordingly, we review the diverse properties of these compounds in aqueous and hydrophobic environments within the framework of the concepts of drug compartmentalization and localization.

Biological effects generally trace with the compartmentalization of a drug and are exemplified by cisplatin, where the anti-cancer effects arise after permeation into the nucleus forming irrepairable adducts with DNA [45]. However, prior to reaching the nucleus, the primary cytotoxic effects are observed immediately after cell uptake and involve the hydrolyzed forms of cisplatin [45]. Although compartmentalization in biology generally refers to placement within specific cellular organelles, in chemistry, the definition is typically broader [46]. In this review, we use the term compartmentalization more broadly. Since we are primarily concerned with whether the drug is found in or at the membrane, the term

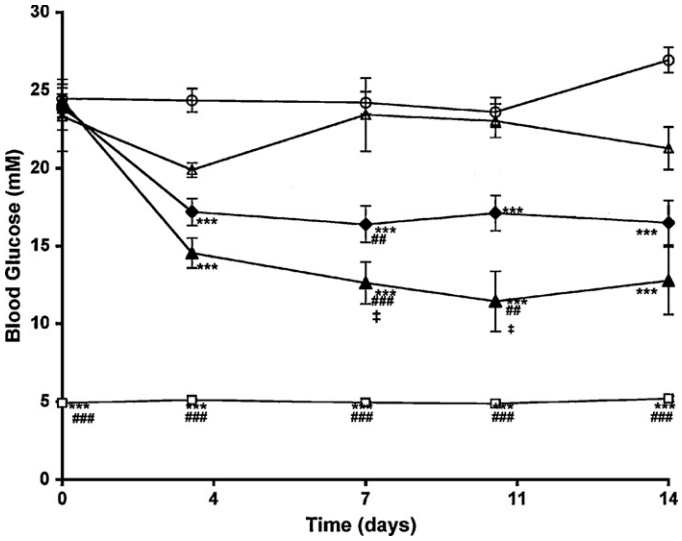


Fig. 3. The effect of vanadium-dipicolinate on hyperglycemia in Wistar rats with STZ-induced diabetes. Compounds were administered in drinking water, and blood glucose was measured for *N* (□, $n = 13$), untreated D (○, $n = 25$), H_2dipic-treated D (△, $n = 6$), $VOSO_4$-treated D (♦, $n = 30$), and $NH_4[VO_2dipic]$-treated D (▲, $n = 5$) animals. Data are presented as the mean standard error of the mean (SEM). $^{***}p \leq 0.001$ vs. the D animals, $^{\#\#}p < 0.05$ vs. the ligand-treated D animals, $^{\#\#\#}p < 0.001$ vs. the ligand-treated D animals. $^{\ddagger}p > 0.05$ vs. the N animals, which means it is statistically indistinguishable from normal. Adapted with permission from Ref. [3].

compartmentalization will also refer to placement of a drug in the membrane. Specifically, the term compartmentalization will describe placement of the V compounds in various environments. Regardless of the exact use of the term, compartmentalization undoubtedly has profound effects on V-based drug action. We hypothesize here that V compound compartmentalization – in the broad sense – can explain the diverse effects including beneficial and toxic effects exerted by V derivatives. We propose that the differences in the effects of V compounds may in part originate from the environment of the compound and that the membrane provides the framework from which the beneficial and malicious effects of V compounds resulted.

2. Interactions of vanadium compounds and specifically $[VO_2dipic]^-$ with proteins

Vanadium compounds interact with a range of diverse proteins [28,29,31,43,47–57]. Only few proteins have specific known functions involving binding of V [54,58–64]. One example is the recently discovered vanabins, abbreviated from *vana*dium *bin*ding proteins, have only been found in tunicates (sea squirts) so far [58]. These proteins bind multiple V ions, generally in oxidation state 4+. Vanabins are thought to be involved in transport of V through the cytoplasm to vacuoles; however, their role is not yet been firmly established [59,60]. V(4+) has also been found as a co-factor in V-containing nitrogenases found in free-living and symbiotic diazotrophs (bacteria), where V replaces molybdenum [61]. In red algae, V is found as a co-factor in haloperoxidases, and in the presence of the vanadate V(5+) co-factor act as a peroxidase [54,62,63]. The activity of these proteins in the absences of vanadate exhibits a dual activity from peroxidases to phosphatases [64]. These proteins have also not yet been found in mammals.

There are numerous effects of V on mammalian biological proteins. Most well-known is inhibition of many phosphorylases including, phosphatases, myosin, ribozymes and phosphodiesterases; for which representative enzymes are shown in Fig. 4 [65]. Vanadium is a potent inhibitor for these enzymes because it is able to form a transition state geometry that resembles the

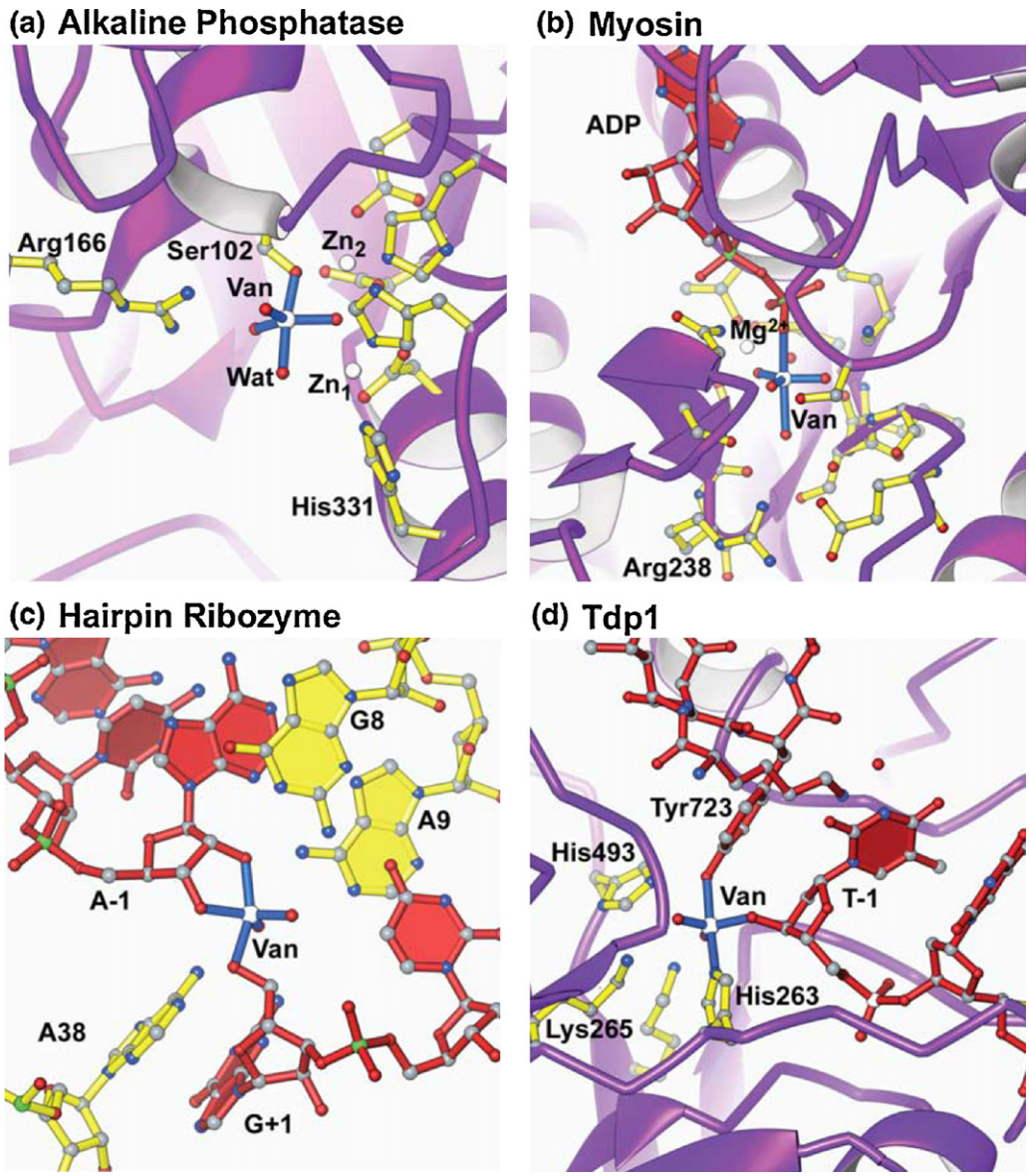


Fig. 4. Selected enzyme–vanadate complexes: (a) Alkaline phosphatase–vanadate complex from PDB ID 1B8J. The protein structure is displayed as a purple ribbon model with amino acid residues displayed as ball-and-stick structures with yellow bonds. The vanadate moiety (Van) is also displayed as a ball-and-stick structure with blue bonds. Carbon atoms are colored gray; nitrogen, blue; oxygen, red and metal atoms are white. Vanadate adopts distorted trigonal bipyramidal geometry with Oγ of the nucleophilic Ser102 residue comprising one apical ligand and a free oxygen atom representing the activated water molecule comprising the other apical ligand. (b) Myosin–ADP–Mg–vanadate structure from PDB ID 1VOM. Coloration for the protein, metal ion and vanadate moieties is the same as in (a) and ADP is displayed as a ball-and-stick structure with red bonds, and phosphate atoms colored green. (c) Hairpin ribozyme–vanadate structure from PDB ID 1M50. Atoms are colored as in (b), with bonds for the ribozyme RNA strand colored yellow and bonds for the substrate RNA strand in red. The vanadate moiety (blue bonds) adopts distorted trigonal bipyramidal geometry with the ribose of nucleotide A-1 contributing one apical and one equatorial ligand and the 5′OH of nucleotide G + 1 contributing the other apical ligand. (d) The quaternary complex around vanadate that mimics the transition state for Tyrosyl-DNA phosphodiesterase from PDB ID 1NOP. Coloration of the enzyme and vanadate moieties is the same as (a) and (b), with the peptide and DNA portions of the substrate analog displayed with red bonds. Adapted with permission from Ref. [65].

transition state of phosphoester and phosphoanhydride hydrolysis [66,67]. Most recognized is inhibition of several protein tyrosine phosphatases, which is believed to have implication for the insulin enhancing effects of these compounds [55,56,68,56,70,71]. The proposed mechanism of action is formation of a high energy intermediate or transition state complex with these protein tyrosine phosphatases. Studies have shown that some V compounds such as peroxovanadium derivatives irreversibly inhibit protein tyrosine phosphatases forming a covalent intermediate that has been observed using mass spectroscopy [56]. Other V compounds such as vanadate form a reversible complex [55,68,71,72]. Specifically V(3+, 4+, and 5+) complexes inhibit several phosphatases including as alkaline, acid, and protein tyrosine phosphatase such as PTP-1b [28,55,67,68,73]. Specifically, $[VO_2dipic]^-$ is a potent phosphatase inhibitor as anticipated by the five-coordinate geometry [67,69,70].

Vanadium is known to compete with iron (Fe) in binding to transferrin (Tf) and another key carrier protein, lactoferrin (Lf), both *in vivo* and *in vitro* [74–78]. VO^{2+} has an affinity for both human serum transferrin and albumin, although the binding to the latter is significantly weaker than the former [78–81]. *In vitro* studies demonstrate decreased Fe delivery to local macrophages in the presence of V [77]. Of the two possible metal binding sites Harris [75] showed that with Tf, most Fe^{3+} binds at "Site A" with high selectivity (~90%) and then at "Site B" during states of excess iron. Additionally, when V or Cr was present, Fe^{3+} and V preferentially bind at site A, while Cr does so at

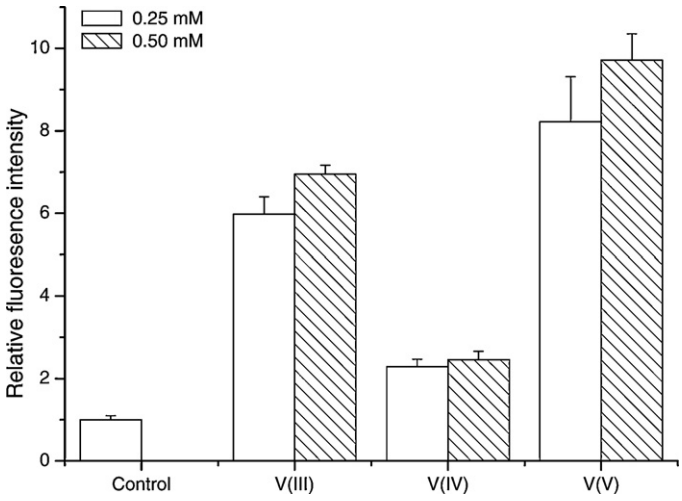


Fig. 5. Quantitation of RONS in Caco-2 cells induced by vanadium complexes using DCFH-DA fluorescent staining (DCFH-DA = 2′,7′-dichlorofluorescein diacetate). All data presented are means ± SD of three measurements. Adapted with permission from Ref. [8].

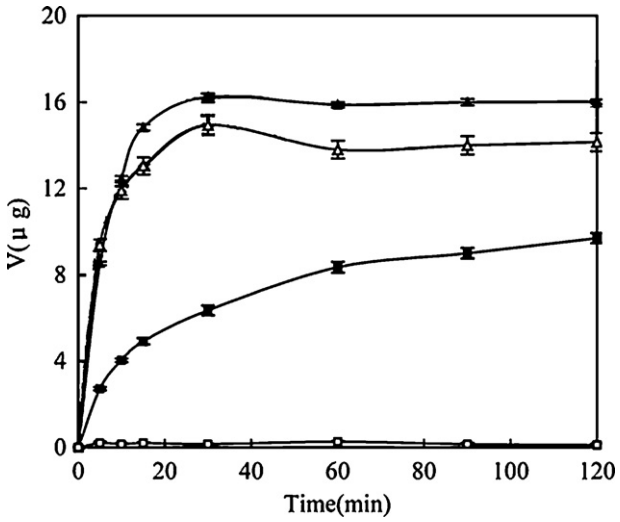


Fig. 6. Kinetic uptake and the DIDS inhibition of the uptake of various vanadium compounds (375 mM) by human erythrocytes. The *x*-axis represents the incubation time and the *y*-axis represents the amounts of vanadium in the cell (C_{in}) at different times. (▲, $VO(ma)_2$; △, $VO(ma)_2$+ DIDS; ■, $NaVO_3$; □, $NaVO_3$+ DIDS) (4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid is abbreviated DIDS). Data represent the means ± SD ($n = 3$). Adapted with permission from Ref. [15].

site B. Thus, while V and Cr each could bind to Tf, V binds with higher affinity, effectively out competing, and thus, blocking Fe^{3+} binding at its preferential site [82]. Recent studies with V in oxidation states 3+, 4+, and 5+ demonstrated that V in all oxidation states can bind to Tf, but that the lower oxidation states bind tightest [80]. Some differences are therefore anticipated for interaction of these proteins with different V complexes. The available studies with bis(maltolato)oxovanadium(IV) BMOV, bis(ethylmaltolato)oxovanadium(IV) BEOV and other complexes, suggest that the metal ion is stripped from the compound upon complexation [44,83–88].

Redox cycling studies of V in cells and in animals are well documented [47]. Studies in animals have shown that both V in 4+ and 5+ forms are observed and continuously recycled, even though such studies are more difficult that corresponding *in vitro* studies [89]. In cells, V(5+) interaction with reductants such as NAD(P)H, glutathione, ascorbate, or catechols has resulted in V(4+) [24,90,91]. Subsequently, V(4+) interaction with oxygen (O_2) or reactive oxygen species (ROS) results in oxidation back to V(5+) [92]. The presence of V(5+) and V(4+) in cells also affects critical enzymes and signal transduction pathways some of which lead to apoptotic cell death pathway [16,24,26,93]. The ability of V to continuously cycle has been suggested as a source of its toxicity [16], as well as its detoxification pathways [8,22]. Fig. 5 illustrates the sharp increase of RONS production in Caco-2 cells upon administration of V-containing compounds; as expected, differences are observed with oxidation state variations in the complex as shown in Fig. 5 [8]. While a constant shuffling of oxidation state for V in cells are anticipated, the fact that differences are observed is indicative that the biological transformations are important because each complex is likely to respond slightly differently. The stability of V is sensitive to environment because ligand coordination can stabilize V in both the V(4+) or V(5+) forms [24,94]. A prime example is the V(4+) stabilization by cellular phosphate anionic ligands which prevents the reduction of O_2/ROS [95] or the effects of redox active peptides [89]. How complexation effects the redox potential of the metal has recently been discussed in detail within the parameters of physiological conditions [96]. Most intracellular V is found complexed as (4+), while (5+) predominates as salt extracellularly [97–99]; this has led to the suggestions that V exits the cell through exocytosis both in the form of salt as V(5+) and complexed as V(4+).

3. Uptake of vanadium compounds and specifically $[VO_2dipic]^-$

Vanadium is readily transported in the blood and high amounts (90–95%) of V in blood are in the plasma fraction, mostly in the form of vanadyl, i.e., V(4+). Salts and several V complexes including BEOV have been studied both *in vitro* and *in vivo* in serum [43,44,83–88,100,101]. Circulatory transport of V in biological systems occurs primarily in association with serum transferrin (Tf), with smaller amounts being associated with albumin and/or low molecular mass (i.e., citrate or lactate) components [22,79]. The degree of involvement of albumin in transport of V is less clear, possibly because some of the *in vitro* studies were done at high concentrations [79,84–86]. In the *in vivo* studies done at lower concentrations [84], no evidence for albumin involvement was found; albumin involvement is likely to vary with the amounts of doses administered. Vanadate has also been found in blood, though at far lower amounts than anticipated considering the reducing environment and significant presence of reductants (e.g., glutathione, ascorbate, and cysteine). Some evidence for V uptake into cells via Tf receptors (TfR) has been reported [102]. In general, Tf serves as the major Fe carrier protein *in situ*, facilitating Fe recycling by transporting Fe from destroyed erythrocytes to the bone marrow for re-use in developing erythrocytes. Administration of BMOV and BEOV resulted in increased amounts of V in bone marrow, consistent with transport and uptake of V through Fe uptake pathways [79]. In addition to protein assisted uptake mechanisms it has been proposed that less soluble V compounds may also use phago- or pinocytosis mechanisms [103].

In salts such as tetravalent (VO^{2+}) or pentavalent (VO_2^+) present in biological systems, transport of V typically takes place through proteins ion carriers. V(4+) is transported quickly into cells and presumably takes advantage of the Fe^{2+}–VO^{2+} analogy [47,104]. The low oxidation state V was also proposed to transport through a mechanism similar to Fe polysulfates [105]. Vanadium(5+), because of the vanadate-phosphate analogy, uses the same anion channels as phosphate for uptake [8,97]. The early studies were carried out in yeast [97] have more recently been confirmed in erythrocytes, along with other cell types, demonstrating that several anion transporters are used Fig. 6 [15]. However, both high oxidation state V

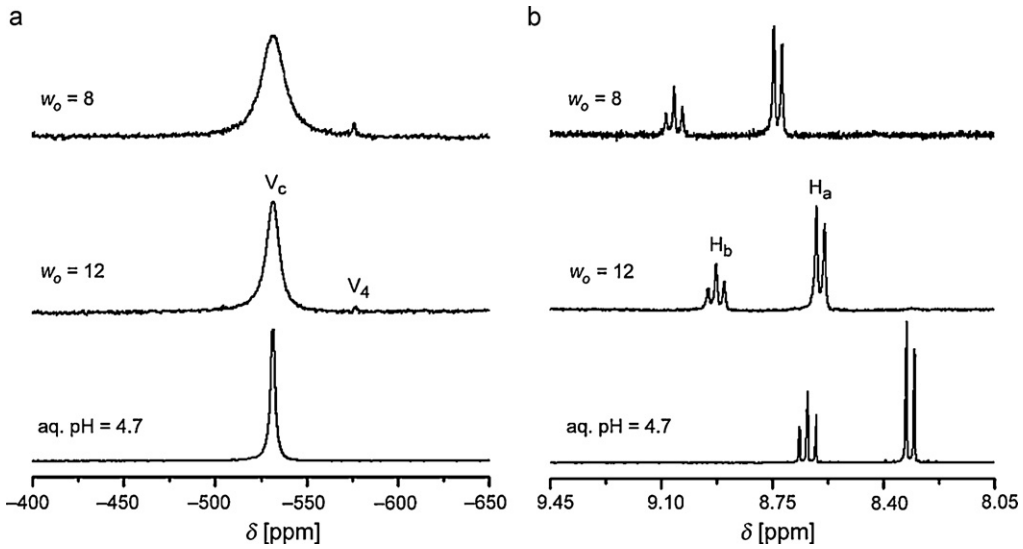


Fig. 7. Stackplot of ^{51}V NMR spectra (a) and ^{1}H NMR stackplot shown, (b) of 100 mM VO_2[dipic]$^-$ in a 1 M AOT/cyclohexane/D_2O (pH 4.7) reverse micelle microemulsion system. Adapted with permission from Ref. [12].

and low oxidation state V more readily enter cells upon complexation [105,106].

Caco-2 cells arranged in a monolayer in a permeability assay show uptake of neutral V coordination complexes though the passive diffusion mechanism [8,14]. Passive diffusion mechanisms of V compounds in cellular systems are consistent with the possibility that membranes play an important role for the action of these compounds. For example, diffusion of BMOV and $VO(acac)_2$ through human erythrocyte membranes was not inhibited by the anion channel inhibitor, 4,4′-diisothiocyanatostilbene-2,2′ disulfonate (DIDS), illustrated in Fig. 6 [15]. In contrast, as illustrated in Fig. 6, vanadate uptake was completely prohibited by anion channel inhibition [15]. These studies demonstrate that V compounds can employ several different mechanisms for entering cells.

It has been proposed, based on previous studies in cells, that V compounds have the potential to reside within the membrane [14,15]. Evidence exists supporting the interpretation that some V compounds affect signal transduction platforms and subsequent signalling events [12,106]. In one simple model membrane system, the ^{51}V and ^{1}H NMR spectra in Fig. 7 showing significant changes in linebroading (^{51}V) and changes in ^{1}H chemical shift indicated an altered environment for $[VO_2dipic]^-$ in this system [12]. These findings were further supported through 2D ^{1}H NMR studies shown in Fig. 8 indicating that $[VO_2dipic]^-$ is present in hydrophobic interfaces [107]. In recent studies, the dipic-ligand showed that a charged form of it is also stable in these environments [10]. This work was performed in the absence of proteins and other variables, and, as such, demonstrates that passive diffusion mechanisms are possible, regardless of the negative charge on the drug. These findings lend support to the possibility that this charged compound and other V compounds can exist in hydrophobic membranous spaces. Importantly, these studies provide precedence that charged V compounds can be thermodynamically favored in hydrophobic environments such as the intermembrane spaces of cells.

4. Vanadium compounds and $[VO_2dipic]^-$ as insulin-enhancing agents in animals and people

Vanadium complexes are known to exert an insulin-enhancing effect in diabetic animals and humans [23,24,27,39–41,43,100,101,108–114]. Some of these compounds, including BEOV, as well as simple salts such as vanadyl sulfate ($VOSO_4$) and sodium vanadate ($NaVO_3$, also called sodium metavanadate), have been used to treat patients in Phase 2 clinical trials [24,40,44,114]. In addition, a wide range of V compounds are known to reduce elevated blood glucose, lipid levels, and in general, alleviate symptoms of diabetes, in streptozotocin (STZ)-induced diabetic Wistar rats [24]. These V-containing compounds lower elevated levels of glucose in diabetic animals as other anti-diabetic drugs. As a result, the symptoms of diabetes, such as increased liquid intake and decreased weight, begin to normalize. However, most remarkable is that these compounds do not lower normal glucose levels in normal animals and thus avoid the potential of

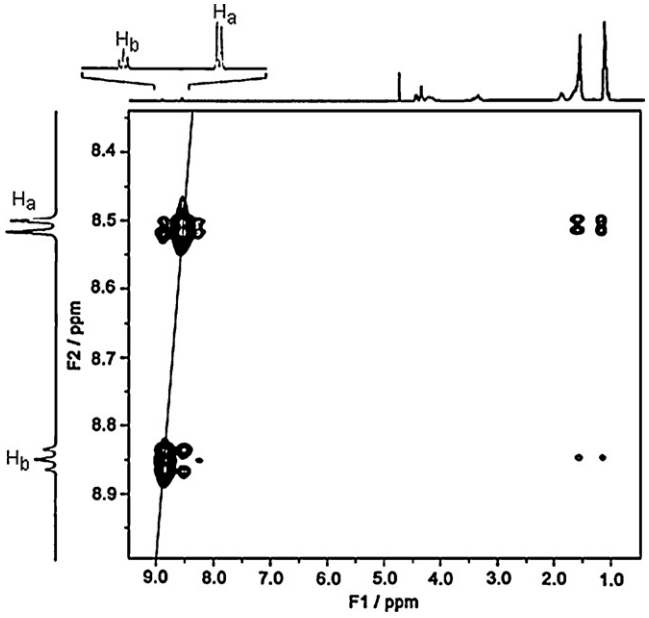


Fig. 8. NOESY spectrum of $[VO_2dipic]^-$ complex in AOT microemulsions in the region showing the negative NOEs arising from interactions between the CH_2 and CH_3 groups in the AOT and $[VO_2dipic]^-$ protons. The scale of F2 domain, showing only the dipic ligand protons are substantially expanded compared to F1, which shows the entire range from AOT methyl groups to $[VO_2dipic]^-$ protons. Microemulsion samples prepared from 1.0 M AOT stock solution in ($^2H_{18}$)isooctane with the 200 mM $NH_4[VO_2dipic]$ in D_2O, pH = 4.5 stock solution at $w_0 = 12$, resulting in an overall concentration of 43 mM $[VO_2dipic]^-$. Adapted with permission from Ref. [107].

hypoglycemia [22,23,49]. Reports exist which show that the effects remain for some time after the patients are no longer taking the drug [115].

Vanadium salts and compounds exert many effects that, combined, result in the observation of insulin-enhancing properties. The most recognized mode of action is the effective inhibition of protein tyrosine phosphatases such as PTP-1B. Inhibition of PTP-1B results in reduced regulation of insulin receptor phosphorylation events leading to increased glucose uptake [116–118]. Recently, the effects of V salts have been characterized using DNA microarray where vanadyl sulfate and oxovanadium(V)4-hydroxydipicolinate normalize the expression of mRNAs [18,22,40]. These studies show that not only were the glucose and lipid metabolism was affected, but that also proteins with roles in oxidative stress, the immune system, and iron metabolism were impacted. The increasing and continued need for treatment of diabetes speaks to the necessity for further investigation of the ability of potential chemotherapeutics to enhance insulin sensitivity [117,118].

Vanadium(4+) complexes represent the largest class of V compounds tested for insulin-enhancing activity [23,24,27,39–41,44,108–114]. Vanadium(4+) complexes such as BEOV have been used in Phase 2 clinical trails [44] although, because of patent protection, most literature exist on the closely related BMOV complex. These complexes have been used in both animal and cell studies showing a significant reduction in diabetic symptoms [3,23]. Studies have shown that reduction of diabetes-induced symptoms such as hyperglycemia and hyperlipidemia, in addition to diabetes-altered genetic effects are decreased with administration of these compounds. More recently, V(5+) and V(3+) complexes have also been found to have insulin-enhancing properties [3,5,8,18,19,119]. Because V(4+) complexes have no charge, they are perceived to be candidates for easy bio-absorption. This fact, combined with the perceived greater stability of these complexes at neutral pH, explains why the V(4+) complexes are popular V insulin-enhancing compounds. However, in the case of the V dipicolinates, for the two derivatives investigated (i.e., the parent and the 4-chloro substituted dipic derivative), the V(5+) complex was the most effective compound in the sense that it was the dipicolinate derivative that produced a statistically-different effect compared to STZ-induced diabetic rats [3,8,19]. Based on the hydrolytic and redox instability of $[VO_2dipic]^-$, the origin of effectiveness for the dipicolinate complexes is not clear, but suggests that some characteristics of these compounds support their biological activities. For this compound it seems likely that transformations are important to its insulin-enhancing action. Therefore, the dipicolinates are a class of compounds that should be particularly well suited for studies investigating compartmentalization of V compounds.

The V(5+) salt, vanadate, and peroxovandadium compounds [120–122] were found early on to be insulin-enhancing. These complexes were subsequently followed by simple coordination complexes of which the oxovanadium(V) dipicolinate was the first [5,18,20,108,123]. Since it was reported, additional V(5+) complexes have been studied including peroxovanadium compounds [122], decavanadate [21], L-glutamic acid gamma-monohydroxamate-vanadium [108,124] and 5-chlorosalicylaldhydeethylenediamineoxovanadium(V) [123]. In BMOV and many vanadium complexes investigated early on [24], the vanadium is in oxidation state 4+; however, the other oxidation states have now also been accepted as insulin-enhancing [8,19]. A few reports in the literature that suggest V compounds have no effects [125] can be attributed to a statistical abnormality with a heterogeneous population in which both responders and non-responders exist [22]. In contrast, amavadin was found to induce some toxic effects, but no insulin enhancing effects [49]. Since amavadin has reversible aqueous redox chemistry, it has been suggested that the redox properties may be important for the compound's biological activities. Vanadium dipicolinate and chloro-dipicolinate complexes were the most effective with the metal in oxidation state 5+ ($[VO_2dipic]^-$ and $[VO_2dipic\text{-}Cl]^-$). Given recently reported chemistry with dipicolinate vanadium complexes in hydrophobic environments [33–35], we describe the known activities of the parent compound, ($[VO_2dipic]^-$) and reconciling it with its fundamental physical and chemical properties. We propose here a framework in which both beneficial and toxic effects can result from the same vanadium compound based on its chemical and biological transformations.

5. Toxicity of vanadium compounds and $[VO_2dipic]^-$

The toxicology for different V(5+) and V(4+) agents, some of which are shown in Fig. 9, has been reported [16,126,127]. However, much less has been reported for the V-dipic complexes. Of the few studies in the literature on this system, most have dealt with clinical parameters affected by V-dipic complexes in diabetic hosts [92]. Though endpoints such as weight loss and renal function were monitered in many studies, these revealed little toxicity. In contrast, at both a cellular and an organ/organ-system level, V(3+), V(4+), or V(5+)-dipic agents are known to impart differential toxicities [8]. In studies with Caco-2 cells obtained from a human epithelial colorectal adenocarcinoma line, changes in viability over a 48-h treatment period showed trends in toxicity potentials with the 5+ changing most and the 4+ changing least (V(5+) > V(3+) > V(4+)), although absolute values of the reported endpoints (i.e., IC_{50}s) did not statistically differ [8]. Specifically, the IC_{50} values reported for $[VO_2dipic]^-$ were close to those of $[VO(ma)_2]$, yet much higher (i.e., less cytotoxic) than for $[VO(acac)_2]$ or metavanadate [14,60]. A relatively greater toxicity from V(3+) compared to the V(4+)-dipic complex was also evident in rats that inhaled either complex 5 h/d for five consecutive days [16,17]. These effects were determined by examination of the animals's lungs and associated local immune responses [16,17]. As in the *in vitro* studies, in comparison to V(3+) and V(4+)-dipic complexes, vanadate again imparted the greater toxic effect *in vivo*.

Though precise mechanisms underlying toxicities of the various V-dipic agents are not known, several potential clues exist. For example, in the Caco-2 studies, treatment with $[VO(ma)_2]$, $[VO(acac)_2]$, and vanadate each resulted in significant increases in formation and intra-cytoplasmic localization of ROS. It is reasonable that these same cells also displayed decrements in trans-epithelial electrical resistance (TEER) that paralleled ROS formation trend patterns among the three agents. In similar studies with dipic complexes in oxidation state 3+, 4+, and 5+, on production of RONS, the cytotoxicity potency patterns were followed. However, unlike with vanadate and the other agents, effects on TEER did not follow the same pattern; the weak RONS inducer V(4+)-dipic-derivative caused as strong a decrease in membrane integrity as its V(5+)-dipic counterpart. These unexpected outcomes with the V-dipic compounds – with respect to membrane integrity and/or cell survival – suggest that the relationship between capacity to induce reactive species and damage to the lipid bi-layer may not be the sole basis for toxicity.

Indeed, in assessing effects of $[VO(ma)_2]$, $[VO(acac)_2]$, and vanadate on erythrocyte membrane fragility, it was suggested that the binding with biomolecules affect the action of the compounds and thereby their potency [15]. Alternatively, the ability of each V agent to impact on levels of cellular reductants not only would affect the cell ability to abrogate any ROS/RONS-induced damage to the inner membrane. However, this mechanism gives rise to the conundrum that the same lack of reductants would lead to mitigation of the

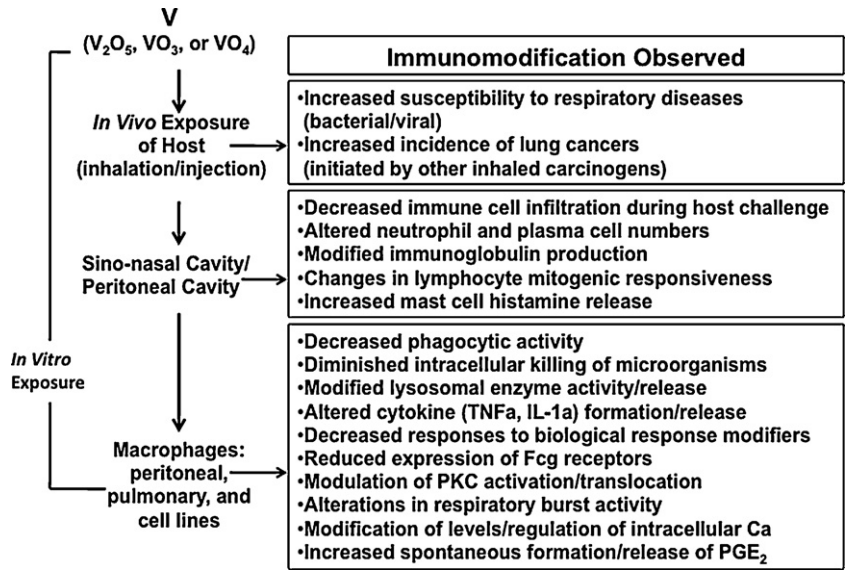


Fig. 9. Known toxic effects from exposure to vanadium compounds *in vivo* and *in vitro* and corresponding effects on the immune system.

activity of a key enzyme involved in initiating ROS/RONS formation in a cell, NADPH oxidase. Of course, many ROS/RONS can be formed non-enzymatically; thus, the mere presence of these metals that can redox shuttle V(5+)/V(4+) creates a scenario for formation of reactive species. This is, of course, particularly possible if the V(5+) or V(4+) is chelated by some unidentified cytoplasmic biomolecule or entrapped in a membrane environment.

Taken together, results of these Caco-2 studies clearly demonstrate that the ligand influences the toxicity of a V agent but, that for any given ligand, the oxidation state/redox behavior of the metal itself ultimately defines its toxic potential. Stated differently, ligand effects are reflected by variations in extent of permeability into (or out of) cells; with the complex redox potential, the amount of oxidative damage to critical cellular macromolecules (proteins, DNA, RNA, etc.) is defined. Thus, the localization of V compounds (in particular, the V-dipics) at the interface of a cell membrane and subsequent interactions with membrane-associated constituents prior to entry into the cytoplasm are potentially critical events in defining toxicity of the agent/drug. As such, the membrane should impact on the degree of interaction with a given V compound and so affect metal/agent penetrance. The particular location of the cell in the body and/or the cell type-associated composition of the membrane are also likely to be important variables to consider in defining the potential efficacy and toxicity of V-based drugs.

6. Chemistry of $[VO_2dipic]^-$ in aqueous solution

The coordination complex $[VO_2dipic]^-$ has been structurally characterized and the vanadium is five coordinate, in a distorted trigonal bipyramidal geometry [128]. The N-atom is in the plane with the cis-dioxo group with the V=O bonds lengths of about 1.6 Å and V–O bonds around 2.0 Å. The V–N bond length is around 2.08 Å, which is a normal V–N bond length even though V-atom coordinates to the pyridine-N atom. These parameters reflect the structural rigidity of this tridentate ligand. Structural characterization of several different salts and of different dipic-derivatives have been reported, with little difference in the anionic coordination complex [4,7,18,129,130].

The $[VO_2dipic]^-$ forms from vanadate and H_2dipic in aqueous solutions ranging from pH 2.0–6.5. Solutions prepared from solid complex or vanadate and ligand are identical within milliseconds of dissolution of complex and ligand. The hydrolytic stability of the complex is highest from pH 3–5 as have been demonstrated both using ^{51}V NMR spectroscopy (Fig. 10a) and ^1H NMR spectroscopy (Fig. 10b) [4]. These studies have also been confirmed using potentiometry (Fig. 10c) [4]. Outside of this pH range, the complex hydrolyzes to form ligand and vanadate in an aqueous environment. The complex stability is sensitive to ionic strength and reduces in the reducing cellular environment. The cyclic voltammogram is irreversible in aqueous solution. The properties for a series of derivatives of this V(V) complex have been characterized and some differences and similarities have been identified; the reader is referred to the original publications for details [4,7,18,129].

Although the complex is stable from pH 2.0–6.5, the complex undergoes chemical exchange because the ligand comes on and off in aqueous solution (Fig. 11). The complex reacts with hydrogen peroxide and then its lability decreases and stability increases [131]. The lability of the parent complex is least near pH 3 and it was considered as an important factor in the compound's mode of action [4]. Other derivatives have been prepared and their chemistries described [4,7,18,129,130]. Several of these compounds have also been tested in animals, and all were found to be insulin-enhancing [3,5,6,18–20]. Nevertheless, none of these novel agents was significantly better than the original parent complex.

The question which species exist after the $[VO_2dipic]^-$ complex is administered is important and key to speculations regarding the mode of action of the complex. In the absence of experimental data, we attempt to answer this question based on properties of the complex and that of others for which pharmacokinetic information is available [44,84–88]. Studies with BMOV and/or have shown that this complex, which is significantly less labile than $[VO_2dipic]^-$, results in the dissociation of the complex [44]. Accordingly, the consensus in the literature would be that the metal and the ligand part ways. However, some of the biological studies have shown that there are statistically significant differences in the results depending on the specific V-dipic complex used [3,6,8,19]. If such differences exist, some form of stabilization must be in effect or differences would not be observed. One possible solution is that the $[VO_2dipic]^-$ complex is trapped, possibly by some form of compartmentalization, which could involve a protein and/or a membrane. Since proteins and membranes are much more hydrophobic environments, it becomes important to consider the properties of these complexes in hydrophobic environments.

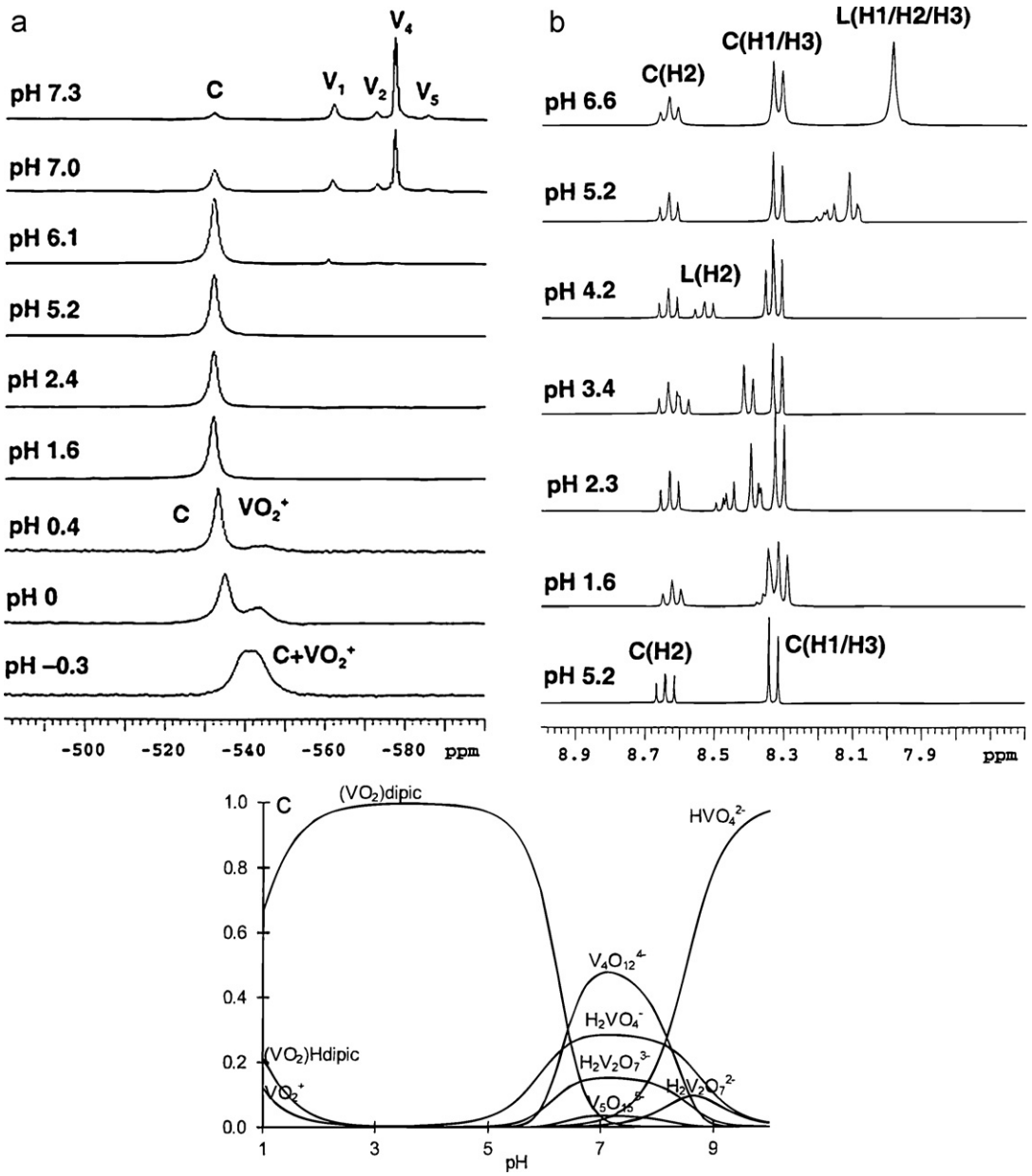
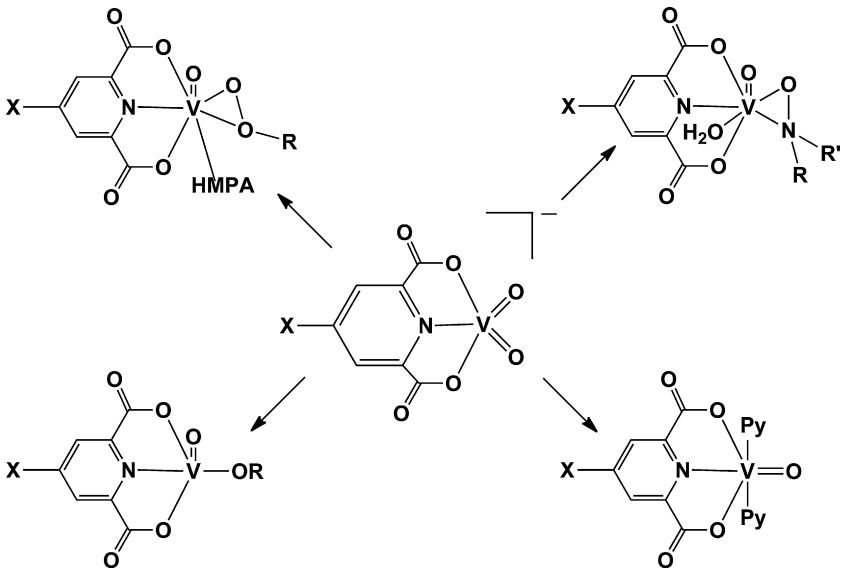


Fig. 10. The ^{51}V NMR spectra of 10 mM $NH_3[VO_2dipic]$ at varying pH (a), ^{1}H NMR spectra of 37 mM $NH_3[VO_2dipic]$ and 31 mM free H_2dipic at varying pH; bottom spectrum is from crystalline $NH_3[VO_2dipic]$ (b) and speciation diagram of 2.00 mM $[VO_2dipic]^-$ in 0.40 M KCl at 25 °C (c). Adapted with permission from Ref. [4].

7. Compatible $[VO_2dipic]^-$ complexes with organic and hydrophobic environments

A number of VO^{3+}-dipic derivatives reported in organic solvents, including peroxo, hydroxylamido, and alkoxovanadium compounds, are shown in Scheme 2. Despite the polarity of the VOdipic unit, these reports demonstrate that derivatives can form in hydrophobic environments even when beginning with the parent $[VO_2dipic]^-$ complex. The first characterized system is the peroxovanadium-derivative, of which the asymmetric peroxovanadium-derivative has the seven coordinate vanadium in a distorted pentagonal bi-pyramidal coordination geometry. This system provides the structural evidence for an asymmetric peroxovanadium compound [131]. Reaction of $VO(OiPr)_3$ with aqueous *tert*-BuOOH and H_2dipic in CH_2Cl_2 resulted a in complex containing a coordinated water or hexamethylphosphoramide (HMPA) molecule depending on the presence of HMPA [131], Schemes 3 and 4. The HMPA adduct is soluble in organic solvents and the water adduct was only soluble in acetonitrile, acetone or water. The water adduct could be converted to the HMPA peroxovanadium derivative demonstrating that the water is exchangeable and that the HMPA adduct is very stable.

The hydroxylamidovanadium complex is a similar system to the peroxovanadium; a range of dipicolinate complexes have been reported, Scheme 5 [129,130,132]. Structural characterization also shows this class of complexes expand the coordination sphere from six to seven by coordination of a H_2O molecule. The stability of this class of complexes has been characterized in detail both in aqueous and organic solutions. The complexes have been prepared by a high yielding two-step synthesis through the $[VO_2dipic]^-$ complex first or a lower yielding one-step pH sensitive process from vanadate, dipic, and hydroxylamine at ambient or lower temperature as illustrated in Scheme 5. The electronic properties of the complexes are very sensitive to the substitution on the hydroxylamines and less so to the substitution on the dipic [129,130]. In aqueous solution, the systems are more stable than the parent complex once formed both



Scheme 2. The types of compounds that have been reported in hydrophobic environment derived from $[VO_2dipic]^-$ complexes.

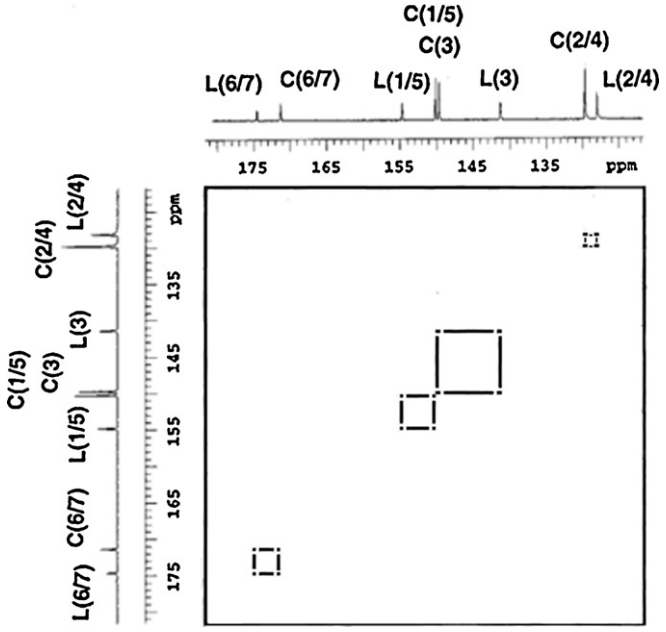
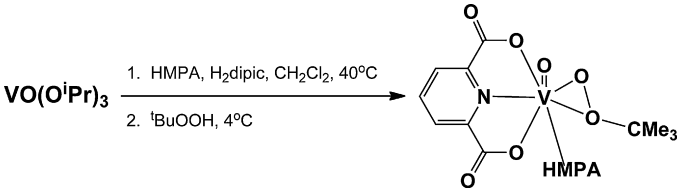


Fig. 11. The ^{13}C EXSY spectrum of the $[VO_2dipic]^-$ complex (687 mM) in the presence of free ligand (581 mM) at pH 6.6 (±0.1). Adapted with permission from Ref. [4].

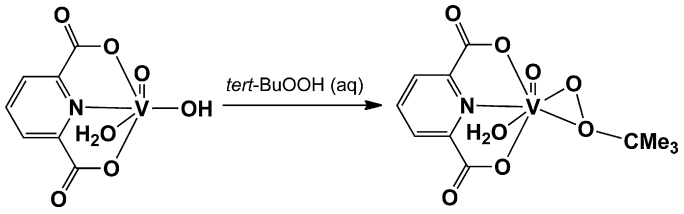
with regard to hydrolysis and with regard to redox chemistry. Less information is available regarding the reactions of these complexes in organic solution, although related systems have been studied and found to undergo hydroxylamine exchange in acetonitrile [132]. Formation of these complexes from the basic components and their resulting stability are important because they document that such complexes can form from the parent $[VO_2dipic]^-$ complex, and once formed, can exist in a hydrophobic environment.

Recently, alkoxide derivatives of the parent $[VO_2dipic]^-$ complex have been reported to form and react in hydrophobic environments. Oxovanadium trialkoxides are well known, including the methyl, ethyl, isopropyl, t-butyl, benzyl, norbornyl and adamantyl derivatives [133–136]. The simplest of these derivatives are prepared from $VOCl_3$ or V_2O_5 through azeotropic distillation. Generally, these complexes associate in organic solvents which can be readily seen by ^{51}V NMR spectroscopy. The most dramatic example is observed for $VO(OCH_3)_3$, where the shifts at sub-millimolar concentration is 100 ppm downfield from more concentrated solutions [133,134,137]. The most commonly used alkoxide derivative, oxovanadium triisopropoxide, is a convenient starting material for preparation of a range of V(5+) compounds and is commercially available. With exception of the alkoxides of the larger alkyl derivatives, such as norbornyl and adamantyl derivatives, these complexes hydrolyze in the presence of water [136]. A wide range of chlorooxovanadium mono and dialkoxides have been prepared and studied [133–144]. Some of these exhibit the same association tendencies as found for the oxovanadium trialkoxides. Many of these alkoxides have been used as synthetic catalysts in organic syntheses.

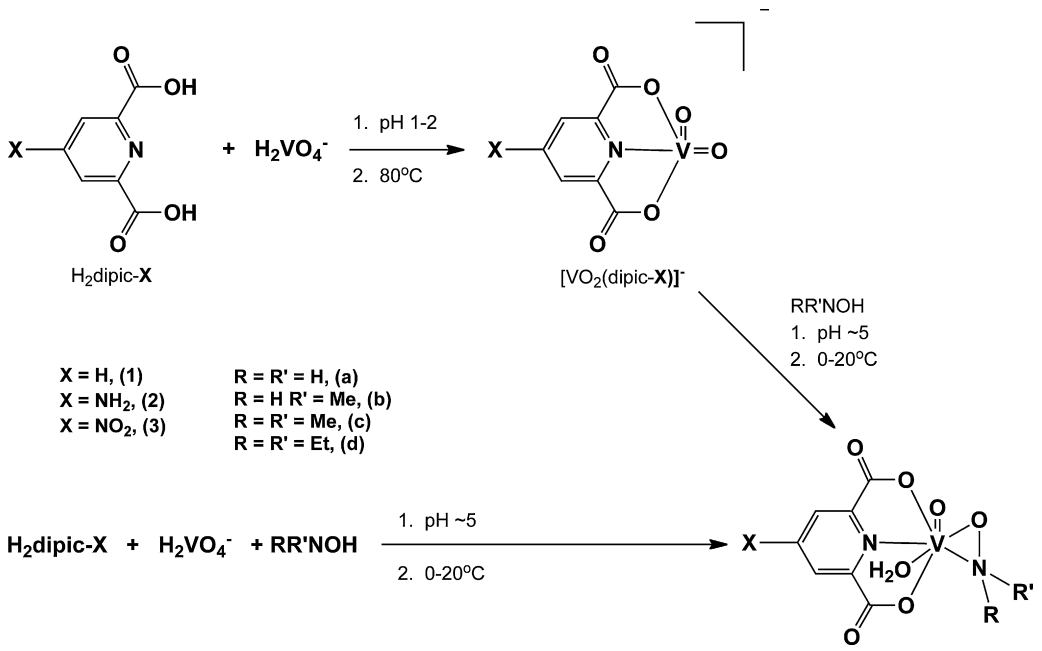
Dipicolinatooxovanadium monoalkoxides such as the ethoxide have been reported, Scheme 6 [145]. Additional complexes are readily formed by alcohol exchange reactions. The literature is in disagreement with regard to the exact nature of these compounds. The original report by Wieghardt [128] described the compound as a protonated $[VO_2dipic]$-complex with a coordinated



Scheme 3. Reaction of $VO(O^iPr)_3$ with aqueous *tert*-BuOOH, H_2dipic and HMPA in CH_2Cl_2 afforded the peroxovanadium compound in a 75% yield [131].



Scheme 4. Reaction of $H[VO_2(dipic)]\cdot H_2O$ (concentrated aqueous solution) with excess aqueous 70% *tert*-BuOOH at 5 °C in 75% yield. Structure was confirmed by elemental analysis [131].



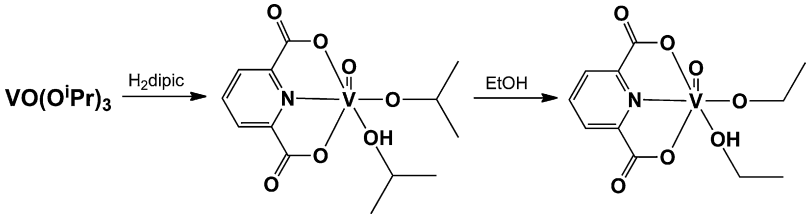
Scheme 5. Two possible routes for preparation of hydroxylamido dipicolinatooxovanadium complexes. The higher yielding two-step reaction (top) or a more sensitive lower yielding one-pot reaction in which redox side-products are more prevalent (bottom), Ref. [129].

alcohol, $H[VO_2dipic]\cdot C_2H_5OH$. This was formed when attempting to recrystallize $H[VO_2dipic]\cdot 2H_2O$ from ethanol. Upon heating at atmospheric pressure and under a nitrogen atmosphere, this complex releases ethanol. The corresponding peroxovanadium complex is sufficiently stable that an X-ray structure could be obtained demonstrating that this complex could withstand the radiation source [145]. The Thorn group represent the oxovanadium(V)dipicolinate complexes as five coordinate oxovanadium(V) alkoxides, in part, because they report that the solvent (alcohol) adduct does not impact the properties of the complex [33–35]. The formation of pinacolate complexes of oxovanadium(V)dipicolinate was achieved by reacting either of the aforementioned V(5+) species [(dipic)VO(OEt)]·EtOH or [(dipic)VO(O^iPr)]·^{i}PrOH with the pinacol in acetonitrile [33]. The structure of this hexacoordinate dipicolinatooxovanadium(V) complex was confirmed by X-ray crystallography. Acetonitrile is a weakly coordinating solvent, and during the crystallization the complex dimerizes. One of the V-atoms remains coordinated with an alcohol whereas the other V-atom has the sixth coordination site occupied by the carboxylate oxygen of the dipic group. These two V-atoms are distinct and should result in two different ^{51}V NMR chemical shifts. Only one chemical shift of −518 ppm was observed suggesting rapid exchange between the vanadium atoms or accidental overlap between the signals [34,35]. This structure is different than a previously-reported dimer prepared from methanol by the Parajon-Costa group. In this complex the two six-coordinate V-atoms are identical and is formed by a diamond core structure by dimerization of the $[VO_2]$ unit [146]. Regardless of the nature of these complexes, these studies demonstrate that a rich redox chemistry is observed a in hydrophobic environment. If a $[VO_2dipic]^-$ complex associates with a membranous environment, such reactions could take place and it becomes important to understand these reactions further.

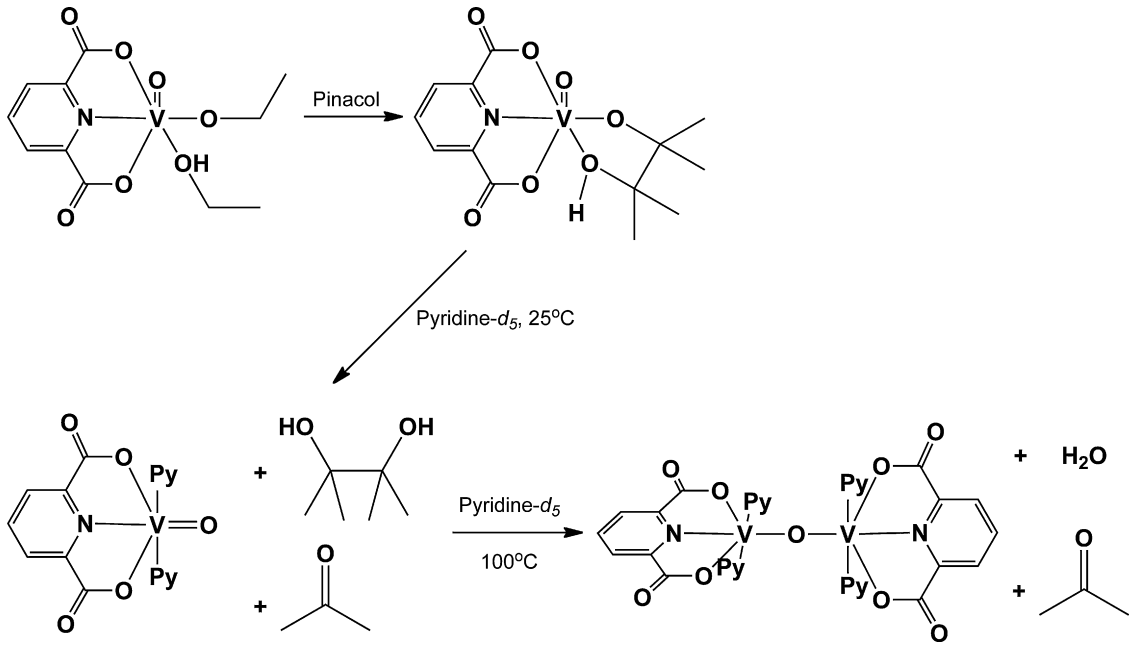
8. Reactions of dipicolinatooxovanadium(V) alkoxides

Dipicolinatooxovanadium(V) alkoxides catalyze the oxidation of lignin via C–C bond cleavage [34,147,148], a particularly hard synthetic challenge. The dissolution of dipicolinatooxovanadium(V) alkoxides in pyridine resulted in adducts that are sufficiently stable to have been isolated and characterized by X-ray crystallography (Scheme 7). The isolation of pyridine coordination adducts provide sufficient support that several V complexes form and can facilitate further reaction. In fact, absence of pyridine coordination was found to significantly decrease similar oxidation reactions.

One of these dipicolinatooxovanadium(V) alkoxides formed from pinacol undergoes redox chemistry, resulting in C–C bond cleavage of the coordinated pinacol [33]. Specifically, by heating the pinacolate complex of oxovanadium dipicolinate in pyridine, a mixture of starting material, C–C bond cleavage products of the pinacol and a bis(pyridine) adduct of oxovanadium(V) dipicolinate were isolated [33]. At ambient temperature, the same reactants formed the bis(pyridine) adduct and corresponding starting mate-



Scheme 6. Formation and alcohol exchange of a dipicolinatooxovanadium alkoxide. $VO(O^iPr)_3$ was combined with H_2dipic in acetonitrile forming the dipicolinatooxovanadium isopropoxide which was confirmed by X-ray crystallography. The isopropoxide was converted to the ethoxide by adding either ethanol or ethanol/acetonitrile solutions, Ref. [145].

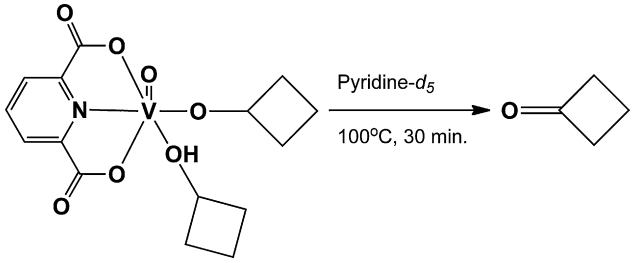


Scheme 7. Dipicolinatooxovanadium ethoxide ($VO(OCH_2CH_3)$(dipic)·$HOCH_2CH_3$) reacts in the absence of water as a catalyst in oxidation reactions such as lignin oxidation. The $VO(OCH_2CH_3)$(dipic)·$HOCH_2CH_3$ complex was reacted with pinacol in pyridine forming a bis(pyridine) adduct at low temperature and a dinuclear complex at high temperature $V_2O(pyr)_2(dipic)_2$ complex, Ref. [33].

rial, but also resulted in oxidation of the pinacol, to the ketone [33–35]. Perhaps the particular reactivity of this complex is due to the coordination of both the alkoxide and an alcohol adduct to the V-atom.

Dipicolinatooxovanadium alkoxides also convert to the $[VO_2dipic]^-$ coordination complex, with loss of both the alkoxide and alcohol. Reaction of either [(dipic)VO(O^iPr)]·^{i}PrOH or [(dipic)VO(Hpinacol)]·solvent with water and pyridine resulted in formation of $[VO_2dipic]^-$ as the pyridinium salt and the alcohol [33]. The coordination of the pyridine to the $[VO_2dipic]^-$ is likely, although as discussed above, only peroxide and hydroxylamine derivatives were observed. Also, the electronic pertubation of the dipic-functionality did result in reorganization of the orbital arrangement of these complexes [130]. ^{51}V NMR studies as a function of pyridine concentration should confirm if such association takes place and was recently reported by the Thorn group [35].

Studies investigating the mechanism of these reactions, specifically regarding whether these reaction take place through a one-electron (V(5+/4+)) or two-electron (V(5+/3+)) redox process, were performed. The Thorn group used the reaction of cyclobutanol [149,150] as diagnostic for this investigation (illustrated in Scheme 8). As described previously, the result of the reaction using cyclobutoxide would indicate a two-electron process if the cyclobutanone resulted and a one-electron process should aliphatic chain product(s) result [151]. The dipicolinatooxovanadium(V) cyclobutoxide produces the cyclobutanone as predicted if a two-electron process were taking place and thus suggests that the V-atom is able to undergo a two-electron transfer reaction under these conditions. Indeed, reactions explored by the pinacol substituted monoethers yielded analogous products [34] suggesting that corresponding chemistry takes place with both protonated and alkylated oxygen. This result is remarkable considering that there is very little precedent for two-electron V(5+/3+) reactions [24,152]. However, the Espenson group questioned the choice of cyclobutanol as it may not be as good a representative alcohol as commonly believed [153] because variations in the product distribution are observed with minor changes in the vanadium complex giving support to alternative explanations. More mechanistic studies on this system have recently been reported [35].



Scheme 8. A two-electron reduction of VO(dipic)(OR)·ROH (R = cyclobutoxide) alkoxide was observed after reaction 100 °C. An average yield of 93% of cyclobutanone with (dipic)$VO(pyr)_2$ as co-product was determined by ^{1}H NMR spectroscopy, Ref. [33].

Recent applications of vanadium catalysts have demonstrated that the studies described above are not unique to dipicolinatooxovanadium(V) complexes [33,34,154–158]. For example, glycine and sarcosine derivatives react in CH_2Cl_2 with $VO(iOPr)_3$ and form, but in the presence of methanol forms a different complex, both of which are effective catalysts for chiral sulfoxide oxidation [154]. Slight modification in ligand or the addition of Lewis base changes the reaction course significantly [155,156]. A second example of how the environment and fine-tuning of a complex allow for improved catalytic effect is reported for the oxovanadium(V) triethanolaminate in combination with pyrazine-2-carboxylic acid [157,158]. This system activates C–H bonds through a water-assisted H-transfer mechanism. The formation of HO• occurs via the addition of H_2O_2 to the V(4+) complex with pyrazine-2-carboxylate [159], but minor changes in this component can significantly change the outcome of the reaction.

In summary, these studies demonstrate the versatility observed in these systems, and support the proposal that hydrophobic environments will dramatically change the reactions of the V–dipicolinate complexes. Thus, should the V-dipic complexes chelate a ligand and be able to interact and penetrate the lipid interface, a different reactivity of the complexes will result. As we

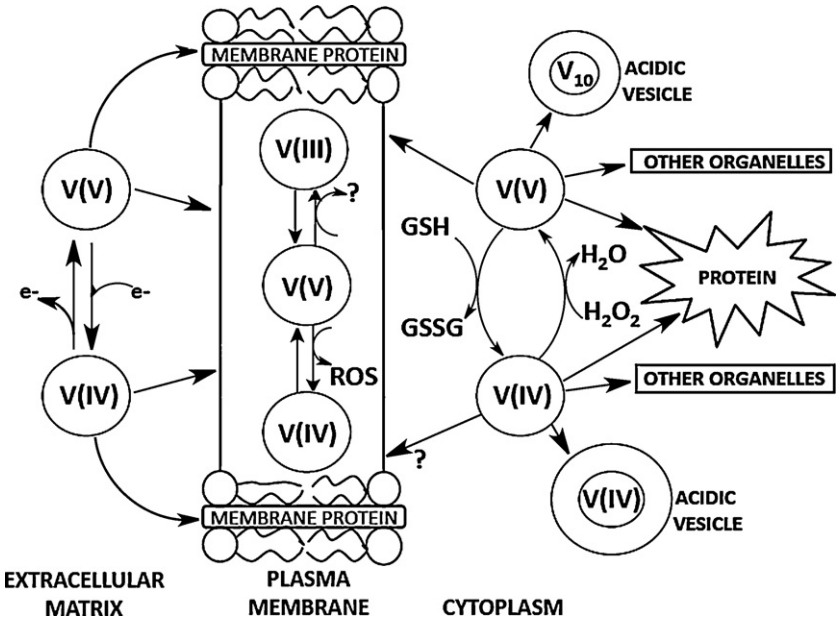


Fig. 12. A proposed pathway for toxic and beneficial responses to vanadium compounds after passive diffusion into the membrane where the hydrophobic membrane environment result in one electron V(4+/5+) redox chemistry and two electron V(3+/5+) redox chemistry. The presence of V(4+) and (5+) in the cytoplasm result in interaction with cellular components in many ways.

present in this manuscript, the biological effects observed by the V-complexes may be a result from the chemical and biochemical transformation that these compounds undergo in the environment in which they are found.

9. How drug environment and compartmentalization might affect action of vanadium compounds

In this manuscript, we suggest that the distribution of vanadium compounds within a cell is critical to their modes of action. Specifically, we propose that a change from a hydrophilic aqueous environment (i.e., as in cytoplasm) to a hydrophobic environment (i.e., as in/near membranes or microenvironments of proteins) governs how any given vanadium compound acts (i.e., beneficial or toxic). Chemistry in organic environments support both one-electron (Figs. 1, 2 and 12) and two-electron processes (Scheme 8 and Figs. 2 and 12) [24,35,152] and thus expands the reactivity of the compound observed in aqueous solution. The compartmentalization is important, because a compound that is less thermodynamically stable in aqueous environments could extend its lifetime by entrapment through compartmentalization in the hydrophobic environment in the membrane. In this review we link the chemistry in both hydrophilic and hydrophobic environments with biological effects of $[VO_2dipic]^-$.

It is generally accepted that vanadium compounds localized in the cytoplasm react with one or more reducing agent constituents, e.g., gluthathione, cysteine, and ascorbate (Fig. 12) [24,95,159]. The accumulation of V(5+) in acidic vesicles resulted in decavanadate formation, which has been observed in yeast and other cellular systems [31,97]. The redox of V(5+) has been associated with transportation of the vanadium into acidic vesicles and with detoxification processes.

Importantly, we have shown that $[VO_2dipic]^-$ and the charged free ligand readily penetrates a membranous surfactant interface residing in a hydrophobic environment [10,107]. Interface penetration takes place despite the high solubility of the complex in aqueous environments and the negatively charged interface. Why this complex is stabilized in this hydrophobic environment is surprising and suggests that some stabilizing forces exist for this system. The recognition that a charged polar compound such as $[VO_2dipic]^-$ has a great affinity for hydrophobic environments is critical for the alternative considerations suggested in this review. Lipid compatibility is not only important for this specific compound, but also demonstrates the concept that charged V derivative and ligands are compatible with hydrophobic membrane environments. As a consequence, the possibility that action of V compounds may involve hydrophobic membrane environments has experimental precedent.

The chemical precedence for conversion of $[VO_2dipic]^-$ to a range of different compounds in organic solvents, Schemes 3–8, show that this compound can form derivatives in hydrophobic environments regardless of its charged precursor. These studies thus support the possibility that the polar $[VO_2dipic]^-$ complex can convert to derivatives that are known to exert very different types of chemistry as described in Schemes 3–8. We propose that V-complexes can form in the membrane environment akin to the reactions taking place in organic solvents. Accordingly, in the presence of as of yet unidentified ligand substitution reactions will lead to controlled two-electron chemistry as is observed for a range of different alcohols. Alternatively, formation of other derivatives that undergo one-electron chemistry is likely to take place, thus result in various radicals and possible formation of ROS and RONS (Figs. 1, 2 and 12). High levels of ROS are likely to result in the deleterious radical chemistry that could signal toxic responses. However, low levels of ROS and RONS have been reported as beneficial, so it is possible that alternative ligands can change the amount of the one-electron reactions or that a two-electron pathway simply serves to reduce the amounts of radicals formed. In both cases the net result could reduce toxicity. Although, it is premature to assign a toxic and a beneficial route for the proposed pathways shown in Figs. 2 and 12; the proposed mechanism does provide alternative modes of action in which membrane interaction are critical to action of vanadium compounds.

10. Concluding remarks

In this review, we have described the chemical and biological properties of a simple and traditional V(5+) coordination complex, $[VO_2dipic]^-$ [128]. The compound is stable in aqueous solution at

acidic pH, but hydrolyzes to form vanadate at neutral and alkaline pHs. At low pHs, the complex forms VO_2^+ and the protonated H_2dipic. The complex is stable in the presence of oxygen, but the vanadium reduces in any reducing cellular environment. The complex is subject to ligand exchange; this occurs both in aqueous hydrophilic as well as a nonaqueous hydrophobic environments and as such result in changes in complex stability and properties [5]. Although we currently do not know the active species, based on the chemical properties of the complex and the different biological activities exerted by this complex, we propose that entrapment of the complex is responsible for the observations. Indeed, compartmentalization of the complex would affect its properties, and provide a rationale for the observations.

Vanadium dipicolinate complexes have been investigated for insulin-enhancing properties; in diabetic animal models, the V(5+) complex generates a statistically different response in treated hosts compared to in untreated controls or diabetic animals [5]. Neither the V(3+) or V(4+) dipicolinate complexes induce a similarly significant response among the diabetic animals. In contrast, the V(4+) in the BMOV complex induced the best response compared to the V(3+) and V(5+) complexes [3]. Since the V(5+) dipicolinate is both unstable hydrolytically and with respect to redox, it is particularly surprising that the $[VO_2dipic]^-$ is so effective. Accordingly, the specific properties of the vanadium dipicolinate complexes are likely important underpinnings to the biological outcomes. We propose that these observations are a result of the compatibility of the $[VO_2dipic]^-$ complex with the membrane environment and its reactivity in hydrophobic environments.

Here, we summarize the known chemistry of $[VO_2dipic]^-$ in organic solvents. Both two-electron and one-electron redox chemistry have been reported [33–35]. We propose that these reactions can take place in analogous hydrophobic environments such as the lipid bi-layers in membranes and in protein interiors. We propose that controlling the chemistry in these environments may induce the observed beneficial effects of vanadium complexes, whereas the less controlled reactions giving rise to high levels of ROS may result in malicious effects of V compounds. At this time, details of the proposed mechanism are ill-defined. We do not know what ligand(s) undergo ligand exchange reaction with the dipicolinate complex to form "the active species". We do not know if the two-electron process is the beneficial process, or simply serves to reduce the ROS levels. However, the hypothesis does provide a more defined alternative mechanistic possibility giving the membrane a role and thus provide explanations to observations not previously explained. Thus, in addition to the inhibition of protein tyrosine phosphatases, changes in redox state of the cell, and interaction with the transport proteins, a testable mechanism has now been proposed in which the membrane plays a key role. Importantly, this proposal (illustrated in Figs. 2 and 12) provides a simple model that explains how the identical complex in some cases induce beneficial effects, and other cases is toxic.

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References

[1] C. Lipinski, F. Lombardo, B. Dominy, P. Feeney, Adv. Drug Deliv. Rev. 23 (1997) 3.
[2] H. Gronemeyer, J.A. Gustafsson, V. Laudet, Nat. Rev. Drug Discov. 3 (2004) 950.
[3] P. Buglyo, D.C. Crans, E.M. Nagy, R.L. Lindo, L.Q. Yang, J.J. Smee, W.Z. Jin, L.H. Chi, M.E. Godzala, G.R. Willsky, Inorg. Chem. 44 (2005) 5416.
[4] D.C. Crans, L.Q. Yang, T. Jakusch, T. Kiss, Inorg. Chem. 39 (2000) 4409.
[5] D.C. Crans, J. Inorg. Biochem. 80 (2000) 123.
[6] D.C. Crans, L.Q. Yang, J.A. Alfano, L.A.H. Chi, W.Z. Jin, M. Mahroof-Tahir, K. Robbins, M.M. Toloue, L.K. Chan, A.J. Plante, R.Z. Grayson, G.R. Willsky, Coord. Chem. Rev. 237 (2003) 13.
[7] J.J. Smee, J.A. Epps, K. Ooms, S.E. Bolte, T. Polenova, B. Baruah, L. Yang, W. Ding, M. Li, G.R. Willsky, A.I. Cour, O.P. Anderson, D.C. Crans, J. Inorg. Biochem. 103 (2009) 575.
[8] Y. Zhang, X.D. Yang, K. Wang, D.C. Crans, J. Inorg. Biochem. 100 (2006) 80.
[9] G.R. Willsky, M. Godzala, P.J. Kostyniak, L. Chi, J.J. Smee, D.C. Crans, The 7th International Symposium on the Chemistry and Biological Chemistry of Vanadium, 2010.
[10] D.C. Crans, A.M. Trujillo, S. Bonetti, C.D. Rithner, B. Baruah, N.E. Levinger, J. Org. Chem. 73 (2008) 9633.
[11] E. Gaidamauskas, D.P. Cleaver, P.B. Chatterjee, D.C. Crans, Langmuir 26 (2010) 13153.
[12] D.A. Roess, S.M.L. Smith, P. Winter, J. Zhou, P. Dou, B. Baruah, A.M. Trujillo, N.E. Levinger, X.D. Yang, B.G. Barisas, D.C. Crans, Chem. Biodivers. 5 (2008) 1558.
[13] J. Stover, C.D. Rithner, R.A. Inafuku, D.C. Crans, N.E. Levinger, Langmuir 21 (2005) 6250.
[14] X.G. Yang, X.D. Yang, L. Yuan, K. Wang, D.C. Crans, Pharm. Res. 21 (2004) 1026.
[15] X.G. Yang, K. Wang, J.F. Lu, D.C. Crans, Coord. Chem. Rev. 237 (2003) 103.
[16] M.D. Cohen, M. Sisco, C. Prophete, L.C. Chen, J.T. Zelikoff, A.J. Ghio, J.D. Stonehuerner, J.J. Smee, A.A. Holder, D.C. Crans, J. Immunotoxicol. 4 (2007) 49.
[17] M.D. Cohen, M. Sisco, C. Prophete, K. Yoshida, L.C. Chen, J.T. Zelikoff, J. Smee, A.A. Holder, J. Stonehuerner, D.C. Crans, A.J. Ghio, Inhal. Toxicol. 22 (2010) 169.
[18] D.C. Crans, M. Mahroof-Tahir, M.D. Johnson, P.C. Wilkins, L.Q. Yang, K. Robbins, A. Johnson, J.A. Alfano, M.E. Godzala, L.T. Austin, G.R. Willsky, Inorg. Chim. Acta 356 (2003) 365.
[19] M. Li, W.J. Ding, J.J. Smee, B. Baruah, G.R. Willsky, D.C. Crans, Biometals 22 (2009) 895.
[20] M. Li, J.J. Smee, W.J. Ding, D.C. Crans, J. Inorg. Biochem. 103 (2009) 585.
[21] M.J. Pereira, E. Carvalho, J.W. Eriksson, D.C. Crans, M. Aureliano, J. Inorg. Biochem. 103 (2009) 1687.
[22] G.R. Willsky, L.H. Chi, Y.L. Liang, D.P. Gaile, Z.H. Hu, D.C. Crans, Physiol. Genomics 26 (2006) 192.
[23] G.R. Willsky, A.B. Goldfine, P.J. Kostyniak, J.H. McNeill, L.Q. Yang, H.R. Khan, D.C. Crans, J. Inorg. Biochem. 85 (2001) 33.
[24] D.C. Crans, J.J. Smee, E. Gaidamauskas, L.Q. Yang, Chem. Rev. 104 (2004) 849.
[25] D. Rehder, Inorg. Chem. Commun. 6 (2003) 604.
[26] A.S. Tracey, G.R. Willsky, E.S. Takeuchi, Vanadium: Chemistry, Biochemistry, Pharmacology and Practical Applications, CRC Press, Boca Raton, 2007.
[27] K.H. Thompson, C. Orvig, J. Inorg. Biochem. 100 (2006) 1925.
[28] D.C. Crans, E.M. Willging, S.R. Butler, J. Am. Chem Soc. 112 (1990) 427.
[29] D.C. Crans, C.M. Simone, A.K. Saha, R.H. Glew, Biochem. Biophys. Res. Commun. 165 (1989) 246.
[30] M. Aureliano, F. Henao, T. Tiago, R.O. Duarte, J.J.G. Moura, B. Baruah, D.C. Crans, Inorg. Chem. 47 (2008) 5677.
[31] M. Aureliano, D.C. Crans, J. Inorg. Biochem. 103 (2009) 536.
[32] M.D. Cohen, in: K. Kustin, J.C. Pessoa, D.C. Crans (Eds.), Vanadium: The Versatile Metal, American Chemical Society, Washington, 2007, p. 217.
[33] S.K. Hanson, R.T. Baker, J.C. Gordon, B.L. Scott, A.D. Sutton, D.L. Thorn, J. Am. Chem Soc. 131 (2009) 428.
[34] S.K. Hanson, R.T. Baker, J.C. Gordon, B.L. Scott, D.L. Thorn, Inorg. Chem. 49 (2010) 5611.
[35] S.K. Hanson, R.T. Baker, J.C. Gordon, B.L. Scott, L.A. Silks, D.L. Thorn, J. Am. Chem. Soc. 132 (2010) 17804.
[36] S. Sunghee, R. Dean Toste, Angew. Chem. 49 (2010) 3791.
[37] C. Limberg, Angew. Chem. 42 (2003) 5932.
[38] V. Conte, B. Floris, Inorg. Chim. Acta 363 (2010) 1935.
[39] S.M. Brichard, Mol. Cell. Biochem. 153 (1995) 121.
[40] A.B. Goldfine, M.E. Patti, L. Zuberi, B.J. Goldstein, R. LeBlanc, E.J. Landaker, Z.Y. Jiang, G.R. Willsky, C.R. Kahn, Metab. Clin. Exp. 49 (2000) 400.
[41] D. Rehder, J.C. Pessoa, C. Geraldes, M. Castro, T. Kabanos, T. Kiss, B. Meier, G. Micera, L. Pettersson, M. Rangel, A. Salifoglou, I. Turel, D.R. Wang, J. Biol. Inorg. Chem. 7 (2002) 384.
[42] K.H. Thompson, C. Orvig, Coord. Chem. Rev. 219 (2001) 1033.
[43] H. Sakurai, in: K. Kustin, J.C. Pessoa, D.C. Crans (Eds.), Vanadium: The Versatile Metal, American Chemical Society, Washington, DC, 2007, p. 110.
[44] K.H. Thompson, J. Lichter, C. LeBel, M.C. Scaife, J.H. McNeill, C. Orvig, J. Inorg. Biochem. 103 (2009) 554.
[45] R.C. Todd, S.J. Lippard, Metallomics 1 (2009) 280.
[46] D.P. Jones, Y.M. Go, Diabetes Obes. Metab. 12 (2010) 116.
[47] N.D. Chasteen, in: H. Sigel, A. Sigel (Eds.), Metal Ions in Biological Systems, Vol. 31: Vanadium and Its Role in Life, Marcel Dekker, New York, 1995, p. 231.
[48] M. Aureliano, R.M.C. Gandara, J. Inorg. Biochem. 99 (2005) 979.
[49] D.C. Crans, Pure Appl. Chem. 77 (2005) 1497.
[50] A.M. Evangelou, Crit. Rev. Oncol. Hematol. 42 (2002) 249.
[51] I.G. Fantus, G. Deragon, R. Lai, S. Tang, Mol. Cell. Biochem. 153 (1995) 103.
[52] I.G. Fantus, E. Tsiani, Mol. Cell. Biochem. 182 (1998) 109.
[53] A. Messerschmidt, R. Wever, Inorg. Chim. Acta 273 (1998) 160.
[54] A. Butler, Curr. Opin. Chem. Biol. 2 (1998) 279.
[55] M. Li, W.J. Ding, B. Baruah, D.C. Crans, R.L. Wang, J. Inorg. Biochem. 102 (2008) 1846.

[56] G. Huyer, S. Liu, J. Kelly, J. Moffat, P. Payette, B. Kennedy, G. Tsaprailis, M.J. Gresser, C. Ramachandran, J. Biol. Chem. 272 (1997) 843.
[57] D.A. Barrio, P.A.M. Williams, A.M. Cortizo, S.B. Etcheverry, J. Biol. Inorg. Chem. 8 (2003) 459.
[58] T. Hamada, M. Asanuma, T. Ueki, F. Hayashi, N. Kobayashi, S. Yokoyama, H. Michibata, H. Hirota, J. Am. Chem. Soc. 127 (2005) 4216.
[59] H. Michibata, M. Yoshinaga, M. Yoshihara, N. Kawakami, N. Yamaguchi, T. Uekil, in: K. Kustin, J.C. Pessoa, D.C. Crans (Eds.), Vanadium: The Versatile Metal, American Chemical Society, Washington, DC, 2007, p. 264.
[60] T. Ueki, M. Satake, K. Kamino, H. Michibata, Biochim. Biophys. Acta, Gen. Subj. 1780 (2008) 1010.
[61] J.P. Bellenger, T. Wichard, A.B. Kustka, A.M.L. Kraepiel, Nat. Geosci. 1 (2008) 243.
[62] J. Littlechild, E. Garcia-Rodriguez, E. Coupe, A. Watts, M. Isupov, in: K. Kustin, J.C. Pessoa, D.C. Crans (Eds.), Vanadium: The Versatile Metal, American Chemical Society, Washington, DC, 2007, p. 136.
[63] A. Messerschmidt, R. Wever, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 392.
[64] W. Hemrika, R. Renirie, H.L. Dekker, P. Barnett, R. Wever, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 2145.
[65] D.R. Davies, W.G.J. Hol, FEBS Lett. 577 (2004) 315.
[66] J.C. Borden, D.C. Crans, J. Florian, J. Phys. Chem. B 10 (2006) 14988.
[67] D.C. Crans, A.D. Keramidas, C. Drouza, Phosphorus Sulfur Silicon Relat. Elem. 109–110 (1996) 245.
[68] X.L. Gao, L.P. Lu, M.L. Zhu, C.X. Yuan, J.F. Ma, X.Q. Fu, Acta Chim. Sin. 67 (2009) 929.
[69] C.C. McLauchlan, J.D. Hooker, M.A. Jones, Z. Dymon, E.A. Backhus, M.A. Youkhana, L.M. Manus, J. Inorg. Biochem. 104 (2010) 274.
[70] C.X. Yuan, L.P. Lu, X.L. Gao, Y.B. Wu, M.L. Guo, L. Li, X.Q. Fu, M.L. Zhu, J. Biol. Inorg. Chem. 14 (2009) 841.
[71] C. Cuncic, S. Desmarais, N. Detich, A.S. Tracey, M.J. Gresser, C. Ramachandran, Biochem. Pharmacol. 58 (1999) 1859.
[72] E. Tsiani, I.G. Fantus, Trends Endocrinol. Metab. 8 (1997) 51.
[73] C.C. McLauchlan, J.D. Hooker, M.A. Jones, Z. Dymon, E.A. Backhus, B.A. Greiner, N.A. Dorner, M.A. Youkhana, L.M. Manus, J. Inorg. Biochem. 104 (2010) 274.
[74] J.P. Harrington, Int. J. Biochem. 24 (1992) 275.
[75] D.C. Harris, Biochemistry 16 (1977) 560.
[76] M.H. Nagaoka, H. Akiyama, T. Maitani, in: M.A. Cser, I.S. Laszlo, J.C. Etienne, Y. Maymard, J.A. Centeno, L. Khassanova, P. Collery (Eds.), Metal Ions in Biology and Medicine, vol. 8, John Libbey Eurotext, Montrouge, 2004, p. 63.
[77] S.P. Doherty, C. Prophete, P. Maciejczyk, K. Salnikow, T. Gould, T. Larson, J. Koenig, P. Jaques, C. Sioutas, J.T. Zelikoff, M. Lippmann, M.D. Cohen, Inhal. Toxicol. 19 (2007) 553.
[78] J.C. Pessoa, I. Tomaz, Curr. Med. Chem. 110 (2010) 3701.
[79] B.D. Liboiron, K.H. Thompson, G.R. Hanson, E. Lam, N. Aebischer, C. Orvig, J. Am. Chem Soc. 127 (2005) 5104.
[80] D. Sanna, E. Garribba, G. Micera, J. Inorg. Biochem. 103 (2009) 648.
[81] D.C. Crans, R.L. Bunch, L.A. Theisen, J. Am. Chem. Soc. 111 (1989) 7597.
[82] H.P. Monteiro, C.C. Winterbourn, A. Stern, Free Radic. Res. Commun. 12–13 (1991) 125.
[83] T. Kiss, T. Jakusch, D. Hollender, A. Dornyei, E.A. Enyedy, J.C. Pessoa, H. Sakurai, A. Sanz-Medel, Coord. Chem. Rev. 252 (2008) 1153.
[84] H. Yasui, A. Tamura, T. Takino, H. Sakurai, J. Inorg. Biochem. 91 (2002) 327.
[85] D. Sanna, E. Garribba, G. Micera, J. Inorg. Biochem. 103 (2009) 648.
[86] D. Sanna, P. Buglyo, G. Micera, E. Garribba, J. Inorg. Biochem. 15 (2010) 825.
[87] H. Esbak, E.A. Enyedy, T. Kiss, Y. Yoshikawa, H. Sakurai, E. Garribba, D. Rehder, J. Inorg. Biochem. 103 (2009) 590.
[88] T. Jakusch, D. Hollender, E.A. Enyedy, C.S. Gonzalez, M. Montes-Bayon, A. Sanz-Mendel, J. Costa-Pessoa, I. Tomaz, T. Kiss, Dalton Trans. 13 (2009) 2428.
[89] D.C. Crans, H. Holst, A.D. Keramidas, D. Rehder, Inorg. Chem. 34 (1995) 2524.
[90] E.J. Baran, Chem. Biodivers. 5 (2008) 1475.
[91] L.A. Minasi, G.R. Willsky, J. Bacteriol. 173 (1991) 834.
[92] A. Stern, X.F. Yin, S.S. Tsang, A. Davison, J. Moon, Biochem. Cell Biol. 71 (1993) 103.
[93] P.S. Chien, O.T. Mak, H.J. Huang, Biochem. Biophys. Res. Commun. 339 (2006) 562.
[94] D.C. Crans, B. Zhang, E. Gaidamauskas, A.D. Keramidas, G.R. Willsky, C.R. Roberts, Inorg. Chem. 49 (2010) 4245.
[95] B.R. Nechay, L.B. Nanninga, P.S.E. Nechay, Arch. Biochem. Biophys. 251 (1986) 128.
[96] D.C. Crans, B. Zhang, E. Gaidamauskas, A.D. Keramidas, G.R. Willsky, C.R. Roberts, Inorg. Chem. 49 (2010) 4245.
[97] G.R. Willsky, D.A. Preischel, B.C. McCabe, Biophys. J. 45 (1984) A76.
[98] M. Barac-Nieto, M. Alfred, A. Spitzer, Exp. Biol. Med. 227 (2002) 626.
[99] S. Elmariah, R.B. Gunn, Am. J. Physiol.: Cell Physiol. 285 (2003) C446.
[100] K. Kawabe, Y. Yoshikawa, Y. Adachi, H. Sakurai, Life Sci. 78 (2006) 2860.
[101] M. Hiromura, A. Nakayama, Y. Adachi, M. Doi, H. Sakurai, J. Biol. Inorg. Chem. 12 (2007) 1275.
[102] S. Tang, B. Lu, I.G. Fantus, Metab. Clin. Exp. 47 (1998) 630.
[103] H. Zaporowska, A. Scibior, Folia Histochem. Cytobiol. 37 (1999) 113.
[104] K. Wang, S. Afr, J. Chem. 50 (1997) 232.
[105] R. Meier, M. Boddin, S. Mitzenheim, K. Kanamori, in: H. Sigel, A. Sigel (Eds.), Metal Ions in Biological Systems, Vol. 31: Vanadium and Its Role in Life, Marcel Dekker, New York, 1995, p. 45.
[106] D.A. Roess, S.M.L. Smith, A.A. Holder, B. Baruah, A.M. Trujillo, D. Gilsdorf, M.L. Stahla, D.C. Crans, ACS Symp. Ser. 974 (2007) 121.
[107] D.C. Crans, C.D. Rithner, B. Baruah, B.L. Gourley, N.E. Levinger, J. Am. Chem Soc. 128 (2006) 4437.
[108] Y. Shechter, I. Goldwaser, M. Mironchik, M. Fridkin, D. Gefel, Coord. Chem. Rev. 237 (2003) 3.
[109] K.H. Thompson, C. Orvig, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems, Vol. 41: Metal Ions and Their Complexes in Medication, Marcel Dekker, New York, 2004, p. 221.
[110] K.H. Thompson, C. Orvig, Dalton Trans. 17 (2000) 2885.
[111] M. Melchior, S.J. Rettig, B.D. Liboiron, K.H. Thompson, V.G. Yuen, J.H. McNeill, C. Orvig, Inorg. Chem. 40 (2001) 4686.
[112] J.H. McNeill, V.G. Yuen, S.T. Dai, C. Orvig, Mol. Cell. Biochem. 153 (1995) 175.
[113] Y. Fujisawa, H. Sakurai, Chem. Pharm. Bull. 47 (1999) 1668.
[114] A.B. Goldfine, D.C. Simonson, F. Folli, E. Patti, C.R. Kahn, Mol. Cell. Biochem. 153 (1995) 217.
[115] G.H. Cros, M.C. Cam, J.J. Serrano, G. Ribes, J.H. McNeill, Mol. Cell. Biochem. 153 (1995) 191.
[116] A.P. Seale, L.A. De Jesus, S.Y. Kim, Y.H. Choi, H.B. Lim, C.S. Hwang, Y.S. Kim, Biotechnol. Lett. 27 (2005) 221.
[117] B.P. Kennedy, Biomed. Pharmacother. 53 (1999) 466.
[118] B. Kennedy, C. Ramachandran, Biochem. Pharmacol. 60 (2000) 877.
[119] K.H. Thompson, Y. Tsukada, Z.M. Xu, M. Battell, J.H. McNeill, C. Orvig, Biol. Trace Elem. Res. 86 (2002) 31.
[120] B.I. Posner, R. Faure, J.W. Burgess, A.P. Bevan, D. Lachance, G.Y. Zhangsun, I.G. Fantus, J.B. Ng, D.A. Hall, B.S. Lum, A. Shaver, J. Biol. Chem. 269 (1994) 4596.
[121] A.P. Bevan, P.G. Drake, J.F. Yale, A. Shaver, B.I. Posner, Mol. Cell. Biochem. 153 (1995) 49.
[122] D.C. Crans, A.D. Keramidas, H. Hoover-Litty, O.P. Anderson, M.M. Miller, L.M. Lemoine, S. Pleasic-Williams, M. Vandenberg, A.J. Rossomando, L. Sweet, J. Am. Chem. Soc. 119 (1997) 5447.
[123] M.J. Xie, X.D. Yang, W.P. Liu, S.P. Yan, Z.H. Meng, J. Inorg. Biochem. 104 (2010) 851.
[124] Y. Shechter, I. Goldwaser, M. Mironchik, H. Tsubery, M. Fridkin, Pure Appl. Chem. 77 (2005) 1617.
[125] V.G. Yuen, P. Caravan, L. Gelmini, N. Glover, J.H. McNeill, I.A. Setyawati, Y. Zhou, C. Orvig, J. Inorg. Biochem. 68 (1997) 109.
[126] F.L. Assem, L.S. Levy, J. Toxicol. Environ. Health, Part B 12 (2009) 289.
[127] J.L. Domingo, M. Gomez, D.J. Sanchez, J.M. Llobet, C.L. Keen, Mol. Cell. Biochem. 153 (1995) 233.
[128] B. Nuber, J. Weiss, K. Wieghardt, Z. Naturforsch. (B) 33 (1978) 265.
[129] J.J. Smee, J.A. Epps, G. Teissedre, M. Maes, N. Harding, L. Yang, B. Baruah, S.M. Miller, O.P. Anderson, G.R. Willsky, D.C. Crans, Inorg. Chem. 46 (2007) 9827.
[130] K.J. Ooms, S.E. Bolte, J.J. Smee, B. Baruah, D.C. Crans, T. Polenova, Inorg. Chem. 46 (2007) 9285.
[131] H. Mimoun, P. Chaumette, M. Mignard, L. Saussine, J. Fischer, R. Weiss, Nouv. J. Chim. 7 (1983) 467.
[132] A.D. Keramidas, S.M. Miller, O.P. Anderson, D.C. Crans, J. Am. Chem Soc. 119 (1997) 8901.
[133] R.K. Mittal, R.C. Mehrotra, Z. Anorg, Allg. Chem. 332 (1964) 189.
[134] A. Lachowicz, K.-H. Thiele, Z. Anorg, Allg. Chem. 431 (1977) 88.
[135] F. Hillerns, D. Rehder, Chem. Ber. 124 (1991) 2249.
[136] D.C. Crans, H. Chen, R.A. Felty, J. Am. Chem. Soc. 114 (1992) 4543.
[137] W. Priebsch, D. Rehder, Inorg. Chem. 29 (1990) 3013.
[138] D.C. Bradley, M.L. Mehta, Can. J. Chem. 40 (1962) 1183.
[139] D.C. Bradley, R.K. Multani, W. Wardlaw, J. Chem. Soc. (1958) 4647.
[140] D.C. Crans, R.A. Felty, O.P. Anderson, M.M. Miller, Inorg. Chem. 32 (1993) 247.
[141] D.C. Crans, R.A. Felty, M.M. Miller, J. Am. Chem. Soc. 113 (1991) 265.
[142] F. Jiang, O.P. Anderson, S.M. Miller, J. Chen, M. Mahroof-Tahir, D.C. Crans, Inorg. Chem. 37 (1998) 5439.
[143] S. Mondal, S. Prasad Rath, S. Dutta, A. Chakravorty, J. Chem. Soc., Dalton Trans. 1 (1996) 99.
[144] J. Salta, J. Zubieta, Inorg. Chim. Acta 257 (1997) 83.
[145] D.L. Thorn, R.L. Harlow, N. Herron, Inorg. Chem. 35 (1996) 547.
[146] A.C. Gonzalez-Baro, E.E. Castellano, O.E. Piro, B.S. Parajon-Costa, Polyhedron 24 (2005) 49.
[147] E.C.E. Rosenthal, H. Cui, K.C.H. Lange, S. Dechert, Eur. J. Inorg. Chem. 23 (2004) 4681.
[148] H. Cui, M. Mummert, S. Dechart, E.C.E. Rosenthal, Inorg. Chem. Commun. 13 (2010) 769.
[149] K. Meyer, J. Rocek, J. Am. Chem. Soc. 94 (1972) 1209.
[150] W.D. Chandler, Z. Wang, D.G. Lee, Can. J. Chem. 83 (2005) 1212.
[151] J. Rocek, D.E. Aylward, J. Am. Chem. Soc. 97 (1975) 5452.
[152] T. Hirao, Chem. Rev. 97 (1997) 2707.
[153] S.L. Scott, A. Bakac, J.H. Espenson, J. Am. Chem. Soc. 114 (1992) 4205.
[154] C. Wikete, P.S. Wu, G. Zampella, L. De Gioia, G.L. Licini, D. Rehder, Inorg. Chem. 46 (2007) 196.
[155] S. Lovat, M. Mba, H.C.L. Abbenhuis, D. Vogt, C. Zonta, G. Licini, Inorg. Chem. 48 (2009) 4724.
[156] M. Mba, M. Pontini, S. Lovat, C. Zonta, G. Bernardinelli, P.E. Kundig, G. Licini, Inorg. Chem. 47 (2008) 8616.
[157] M.V. Kirillova, M.L. Kuznetsov, V.B. Romakh, L.S. Shul'pina, J. da Silva, A.J.L. Pombeiro, G.B. Shul'pin, J. Catal. 267 (2009) 140.
[158] G.B. Shul'pin, Y.N. Kozlov, G.V. Nizova, G. Suss-Frank, S. Stanislas, A. Kitaygorodskiy, V.S. Kulikova, J. Chem. Soc., Perkin Trans. 2 (2001) 1351.
[159] D.C. Crans, B. Baruah, E. GaidamauskaS, B.G. Lemons, B.B. Lorenz, M.D. Johnson, J. Inorg. Biochem. 102 (2008) 1334.