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Vanadium(V) complexes in enzyme systems: aqueous chemistry, inhibition and molecular modeling in inhibitor design

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Abstract

Vanadate in aqueous solution is known to influence a number of enzyme-catalyzed reactions. Such effects are well known to carry over to living systems where numerous responses to the influence of vanadium have been well-documented; perhaps the most studied being the insulin-mimetic effect. Studies of the aqueous chemistry of vanadate provide an insight into the mechanisms by which vanadate affects enzyme systems and suggests methods for the elucidation of specific types of responses. Studies of the corresponding enzymes provide complementary information that suggests model vanadate systems be studied and provides clues as to functional groups that might be utilized in the development of selective enzyme inhibition. The insulin-mimetic effect is thought by many workers to originate in the effectiveness of vanadium as an inhibitor of protein tyrosine phosphatase (PTPase) activity. One, or more PTPases regulate the phosphotyrosine levels of the insulin receptor kinase domain. Appropriate ligands allow modification of the reactivity and function of vanadate. For instance, although the complex, $((\text{CH}_3)_2\text{NO})_2\text{V}(\text{O})\text{OH}$, is not quite as good an inhibitor of PTPase activity as is vanadate, it is much more effective in cell cultures for increasing glucose transport and glycogen synthesis. Studies of the chemistry of this complex provide an explanation of the efficacy of this compound as a PTPase inhibitor that is supported by computer modeling studies. Computer calculations using X-ray data of known PTPases as a basis for homology modeling then suggests functionality that needs to be addressed in developing selective PTPase inhibitors. © 2001 Elsevier Science B.V. All rights reserved.

The seminal paper of Lindquist et al. [1] in 1973 describing the inhibition of ribonuclease by vanadate in the presence of uridine, represented a critical turning point in the efforts to understand the origin of the biological influences engendered by vanadium compounds. Subsequent to this work, the demonstration of the insulin-mimetic effects of simple vanadium(IV) and vanadium(V) salts in live animals provided the impetus that still drives much of the research into development of vanadium compounds that have a potential therapeutic value.

Despite the demonstrated efficacy of various compounds of vanadium in enzyme activation and inhibition, the question still arises as to whether or not vanadium is effective because of its special chemical properties so that other metals, will not serve as well. In certain aspects of vanadium biochemistry, the answer seems clear that, yes, it is the properties of vanadium itself that makes it unique in

biochemistry. In the simplest role of vanadium in enzyme activation this seems unquestioned. For instance, glucose in the presence of glucose-6-phosphate dehydrogenase and vanadate forms gluconic acid [2]. Glucose-6-phosphate is the normal substrate for this enzyme but it has been demonstrated that alcoholic groups in the presence of vanadate, rapidly and reversibly form vanadate esters [3,4] so that glucose-6-vanadate will occur in solution along with other vanadate derivatives that do not serve as enzyme substrates. The charge state and size of the vanadate grouping does not markedly differ from that of the corresponding phosphate. Indeed, the phosphate group can be simply thought of as handle utilized by the enzyme to allow it to properly position the substrate and then promote a chemical reaction in a remote location. In the presence of vanadate, a number of phosphate-metabolizing enzymes function in a similar manner to glucose-6-phosphate dehydrogenase. Such enzymes include epimerases, mutases and isomerases [5,6]. For these enzymes, the role of vanadate is to convert a compound into a form that is recognized by the enzyme so that chemical reactions can occur.

The role of vanadate changes markedly in those cases

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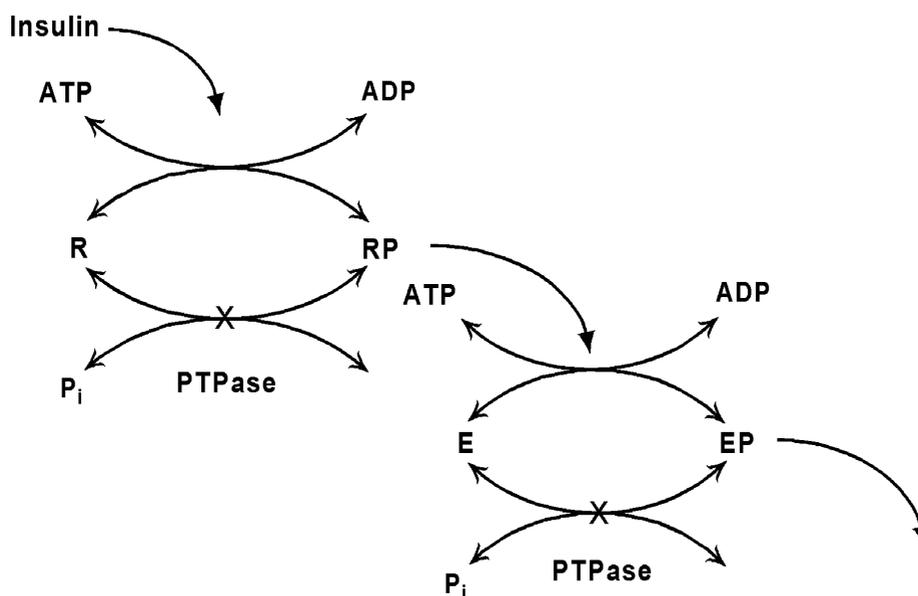
where an enzymatic reaction occurs at a phosphate group, as for instance in phosphate hydrolysis. In such a case, the enzyme is structured in a manner that enables it to provide the energy needed to compensate for the requirements of forming a phosphate transition state. In this case, a similarly structured vanadate requires considerably less energy. Evidence that is available for several enzyme systems suggests that vanadate requires in the order of half of the corresponding energy required by a phosphate to reach the transition state [7,8]. There are a number of reasons why this may be so, including such things as the longer V–O bond lengths compared to phosphate (about 10%), weaker hydrogen bond formation and possibly just an overall inability to accurately mimic the phosphate transition-state structure without a substantial energy input.

One of the exceptionally good ligands for complexation of vanadate is hydrogen peroxide. This complexation reaction has been the subject of investigation over many years. One impetus for this study was the finding that some vanadium hydrogen peroxide complexes are excellent insulin mimetics [9,10]. The mechanism by which the peroxovanadium complexes function is complicated and not clearly understood but the evidence suggests that the activity is triggered by an irreversible inactivation of protein tyrosine phosphatase (PTPases) activity [11]. The inactivation is achieved through oxidation of the sulphur of the essential active site cysteine. Such a cysteine is common to all the PTPases.

A common thread arising from the few characterizations that have been carried out is that peroxovanadate and other vanadate-based insulin-mimetic compounds cause a marked increase in the phosphorylation state of a number of tyrosyl proteins, including the insulin receptor and MAP kinases [11,12]. Scheme 1 depicts how this might happen

in a multistage process that functions through inhibition of PTPase activity. In this scheme, R refers to the receptor and E can be any enzyme including a second receptor. In cases where autocatalytic activity occurs, as in the insulin receptor, there will be a cascade and a rapid build-up of active enzyme, RP. Without selective PTPase inhibitors, there is little or no selectivity of influence on the various reactions of the type depicted in Scheme 1.

It is quite clear that there are signaling pathways that utilize the phosphorylation state of tyrosine residues and that there are a variety of regulatory mechanisms [13]. For expression of the insulin-mimetic effect, is it sufficient that only one enzyme, possibly the insulin receptor, be activated or is it necessary that there are several simultaneous activations? There is as yet no answer to this question. One way to approach the problem is to ascertain the results of using selective phosphatase inhibitors. This is a non-trivial problem that is complicated by the fact that the biological substrates for many PTPases are not known, and there are many PTPases [14]. Additionally, the detailed structures of most of the PTPases are not known. Accumulating evidence strongly suggests that the insulin receptor kinase domain is a substrate of the PTPase, PTP1B [15,16]. PTP1B, has an active site architecture that is not significantly different from other PTPases phosphatases. Indeed this is a characteristic of this class of enzymes [17]. However, since there are a variety of PTPases it may be deduced that there are a number of substrates. Evidently, then, it is necessary to look outside the active site region of the enzyme in order to obtain information about how selectivity of function might be attained. In the cases where a crystallographic structure is available, this a relatively straightforward task. In other suitable cases, modeling techniques can provide the requisite information.



Scheme 1.

This has been demonstrated for some PTPases of interest. The D1 phosphatase domains of leucocyte antigen-related (LAR) and common antigen-45 (CD45) have been studied in detail and their structures compared and contrasted with that of PTP1B [18]. LAR, like PTP1B, has been associated with the function of the insulin receptor [19] while CD45 is critical to T-cell activation [17] and is important in autoimmune diseases. For these three enzymes there are significant differences in the surface surrounding the active site. These differences include both structure variations and surface charge. The changes arise from the different amino acids that occupy corresponding positions in the enzyme. For instance a prominent surface arginine in PTP1B is replaced by a neutral valine in CD45 while in LAR both the arginine and an adjacent glutamate are replaced with neutral amino acids, an alanine and an asparagine [18]. These, and other surface recognition elements, then provide a viable resource that can be utilized in the development of selective PTPase inhibitors.

There is no way to make use of these surface elements with common vanadium-based PTPase inhibitors such as vanadate and pervanadate since, with these compounds, the inhibition arises from interactions within the active site cleft and the active site architecture is quite invariant within the family of PTPases. Other compounds, such as maltolato vanadium(IV) and similar complexes, might act only as carriers of the vanadium so that the ligand is released as the inhibition process progresses. This however, may well be an overly simplistic explanation of their function.

A recently discovered potent inhibitor of PTPase activity is the hydroxylamine complex, bis(N,N-dimethylhydroxamido)hydroxovanadate (VL_2). This compound has an inhibition constant of about $1 \mu\text{M}$ for both PTP1B and LAR-D1 [20]. Structurally, this compound is similar to bisperoxovanadate [21,22] but is not a good oxidizing agent and inhibits PTPase activity in a reversible manner. It also has a fully reversible function in cell cultures [23]. In fibroblast cell cultures it is about ten times as potent as vanadate in effecting glucose transport and about forty times as effective in promoting glucose incorporation into glycogen. In the JURKAT line of cancerous T-cells, it is highly effective while vanadate generates no response. The nature of the actual inhibiting species is not known with certainty. The spontaneous formation of complexes of vanadate and N,N-dimethylhydroxylamine (here referred to collectively as DMHAV) in aqueous solution is highly favored compared to hydrolysis. Hydrolysis is quite slow as can be readily ascertained by dissolution studies of the crystalline material [21] and can effectively be prevented by inclusion of a small amount of DMHA in the solution. Reaction with such hetero ligands as dithiothreitol or cysteine is fast and these bidentate thiolate-containing ligands readily form complexes with VL and VL_2 to give mixed ligand products [24].

Fig. 1 reveals some of the complexity inherent in

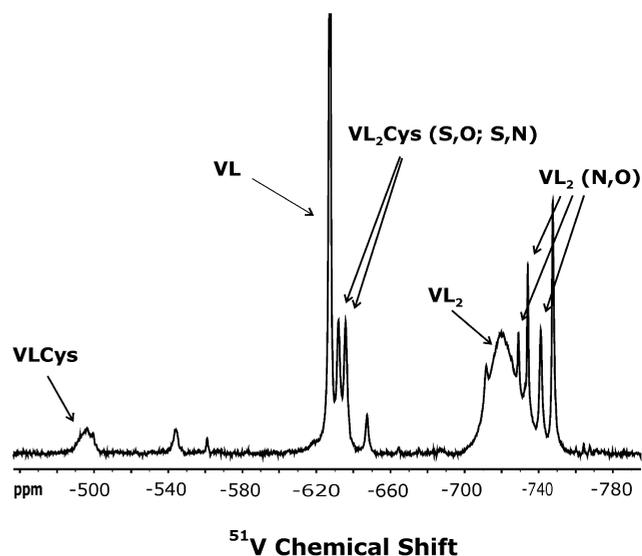


Fig. 1. ^{51}V NMR spectrum of vanadate in the presence of N,N-dimethylhydroxylamine (L) and cysteine (Cys). Conditions of the experiment: total vanadate, vanadate, 5 mM; N,N-dimethylhydroxylamine, 24 mM; Cys, 30 mM.; ionic strength, 1.0 M with KCl; pH 8.54.

understanding the aqueous chemistry. The reaction of vanadate in the presence of N,N-dimethylhydroxylamine and cysteine (Cys) yields a number of products of varying stoichiometries. Both cysteine–vanadate complexes (Fig. 2) and mono and bis hydroxylamine–vanadate complexes [21,25] are formed. Further reaction leads to the formation of mixed ligand complexes. Such complexes include VLCys and VL_2 Cys complexes of variable structure [24].

The sulphur atom in the coordination sphere causes a large downfield shift in the ^{51}V NMR signal position. Thus VL_2 Cys (both S,O and S,N bidentate coordination) give signals about 100 ppm to low field of VL_2 while the signals from VL_2 Cys (N,O coordination) is similar in chemical shift to VL_2 . Similarly, the VLCys NMR signals are about 120 ppm to low field of the VL signal. The types of products formed and demonstrated by Figs. 1 and 2 reveal that V, VL and VL_2 undergo chemical reactions with the same types of reactive groups that are found within the

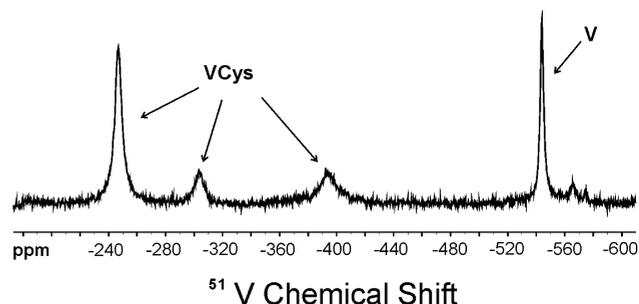


Fig. 2. ^{51}V NMR spectrum of vanadate in the presence of cysteine. Vanadocysteine complexes provide signals at -248 , -305 and -395 ppm. Conditions of the experiment: vanadate, 5.0 mM; cysteine, 60 mM; pH 8.54; KCl, 1 M. Reduction of vanadate occurs under these conditions with the solution becoming green within 15 min or so.

active site of PTPases. Such chemistry is in keeping with the inhibition of protein tyrosine function by vanadate and by DMHAV and suggests that such reactions occur within the active site pocket of these enzymes.

Attempts to experimentally discover whether the mono or the bis ligand dimethylhydroxylamine complex is the functional inhibitor have not proven to be successful. The fact that both VL₂ and the dithiothreitol (T) complex, VL₂T, are inhibitors [20] strongly suggests that one of the hydroxylamine ligands is lost during the inhibition process. It is difficult to see how there would otherwise be room within the active site region. Computer modeling suggests that VL₂ itself, fits well into the active site cleft but the vanadium is not close enough to the sulphur to form a V–S bond. Hydrogen-bonding and hydrophobic stabilization interactions are, however, present in such a binding situation and these together might possibly account for the inhibition by this complex.

In order for the vanadium center to come within a reasonable interaction distance of the sulphur of the active site cysteine, molecular modeling suggests that one N,N-dimethylhydroxylamine ligand must be displaced from the complex. The formation of complexes such as VLCys (Fig. 1) and VLT [24] shows that the appropriate products can be formed. The loss of one ligand in an active site interaction is therefore quite likely. In this event, modeling suggests the inhibitor is oriented as depicted in Fig. 3. For this structure the V–S (2.23 Å, for cys215) and V–O (1.97 Å, for ser216; 2.16 Å, for H₂O) bond lengths are well

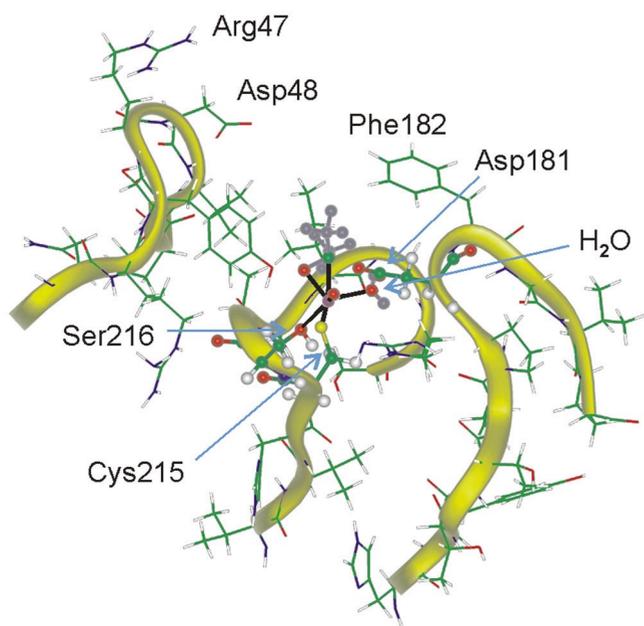


Fig. 3. Representation of N,N-dimethylhydroxamidovanadate (VL) bound in the active site of PTP1B as obtained from molecular modeling. Various active site residues are identified. Some distances of interest: V–Ser216, V–O 1.97 Å; V–Cys215, V–S 2.23 Å; V–H₂O, V–O, 2.16 Å, Asp181–H₂O, O–O 2.67 Å; Asp181–H₂O, O'–N, 2.74 Å; Asp181–Lys120, O–N, 2.68 Å.

within the corresponding distances for similar types of atoms in known compounds. O–O and O–N interatomic distances in the order of 2.7 Å from active site sidechain residues to the complexed water molecule (Fig. 3) suggest the presence of strong stabilizing hydrogen bond interactions.

If Fig. 3 provides a reasonable representation of the binding of VL in the active site pocket of PTP1B then one of the methyl groups of the inhibitor is directed out of the active site and as a consequence is playing a minor role in the inhibition process. Because of this, the methyl group is amenable to chemical modification without that modification having a significant influence on the inhibition process. In fact, the methyl group is in a position that is highly suitable for replacement with functional groups that reach out to the surface recognition elements. Arginine47 is the most prominent of these surface groups for PTP1B but, as is evident from Fig. 3, aspartate48 is also accessible. In CD45, the arginine has been replaced by a valine while in LAR the arginine and aspartate locations are occupied by alanine and asparagine, respectively. The potential for use of these surface elements in the development of selective inhibitors of greatly enhanced potency can then be addressed in a systematic manner. Since the full power of organic synthesis is available for generating replacement groups virtually at will, it seems that hydroxylamine ligation can be combined with a powerful alkyl prosthetic group upon which to hang a template of substituents for investigating these three enzymes. With the availability of more structures, either from modeling or X-ray diffraction studies, PTPase activity can be studied in a systematic manner. This is critical for looking at potential applications such as therapeutic intervention in autoimmune diseases and the insulin-mimetic effect. Doubtless, the characterization of the function of the many other phosphatases will allow a significant expansion of these possibilities.

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