Minireview

The power of vanadate in crystallographic investigations of phosphoryl transfer enzymes

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Abstract The formation of transition state mimics of phosphoryl transfer reactions with the metal oxoanion vanadate is a powerful technique in macromolecular crystallography. The tendency of vanadate to form pentacovalent complexes exhibiting trigonal bipyramidal geometry makes this compound a close approximation of the transition state for such reactions. In many cases, vanadate complexes provide the most accurate visualization of the transition state that can be reasonably achieved. A survey of the Protein Data Bank reveals that a relatively small number of structures (39, representing 23 unique proteins) include vanadate, yet these structures represent four of the six E.C. categories of enzymes, and were obtained in crystals with pH values ranging from 5.0 to 7.8. Vanadate has additional advantages over other compounds such as aluminum fluoride, beryllium fluoride and nitrate used for visualization of transition state mimics in that vanadate readily forms covalent bonds with a variety of ligands and has produced a wider variety of transition state mimics. Given the hundreds of crystal structures that have been solved for phosphoryl transfer enzymes, it is surprising that vanadate has not been used more frequently for visualization of transition state analogs. We propose that an opportunity exists for vanadate to become a more commonly utilized component of the macromolecular crystallographer's toolbox.

Keywords: Transition state analog; Enzyme; Vanadate; Pentacoordination

1. Introduction

The transition metal oxoanion vanadate (VO_4^{3-}) is a close structural and chemical mimic of phosphate. In addition, vanadate can be easily derivatized in aqueous solution with a variety of ligands and tends to adopt a pentacoordinate, trigonal bipyramidal geometry around the central vanadium (V) atom. These features have led to the proposal that vanadate can act as an analog for the conformation of the phosphate group at the transition state expected for phosphoryl transfer [1,2]. As would be expected for such a transition state mimic, vanadate is a potent inhibitor of many phosphoryl transfer enzymes [3,4]. This mimicry has been observed many times in X-ray crystal structures of enzymes complexed with vanadate. These structures have provided a wealth of insight into catalytic mechanism and substrate binding modes. The present review will focus mainly on technical and scientific aspects of the use of vanadate in macromolecular crystallography.

Although the concept of vanadate as a phosphoryl transfer transition state has become very widely accepted [5], it should be noted that this view has been critically evaluated [6,7]. Theoretical studies [6] concluded that V–O bonds are considerably less polar (smaller electrostatic potential) than expected for the corresponding P–O bonds in a true transition state. Additionally, bond angles in enzyme–vanadate complexes have been observed to differ from the corresponding predicted bond angles in ideal trigonal bipyramidal transition states. For example, although the angle between the two apical bonds in the transition state should be 180°, distorted angles of as little as 150.5° have been observed [8]. Another consideration is that phosphoryl transfer is thought to proceed via different mechanisms in different enzymes. Monoester hydrolysis is often thought to occur by a "dissociative" mechanism, where the bond between phosphorus and substrate completely breaks before the bond to the nucleophile forms, whereas phosphodiester cleavage is thought to occur by a more "associative" mechanism where a partial bond forms between phosphorus and the nucleophile before the bond to the substrate is completely broken [9]. This view of phosphoryl transfer mechanisms, however, is still an active area of debate [10,11]. At any rate, vanadate has been observed to form pentacoordinate complexes with a variety of enzymes and may provide information about transition states in either dissociative or associative cases.

Regardless of any remaining controversies, vanadate remains an excellent practical option for approximation of phosphate transition states. The closest chemical approximation of such a transition state is a class of pentacovalent phosphorus compounds known as phosphoranes. Unfortunately, hydroxy phosphoranes that are analogous to the transition state for ester or phosphodiester hydrolysis are generally unstable compounds. Commonly, stable phosphoranes can only be obtained in cases where several ester linkages stabilize the complex [12]. A survey of phosphoranes in the Cambridge Structural Database reveals that the database

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contains no structures of pentacovalent phosphorane structures with fewer than four non-hydroxy ligands and only a handful of structures that possess a single hydroxy ligand. A phosphorane intermediate bound to an enzyme has only been visualized serendipitously in two unusual crystal structures (in phospholipase D, PDB code 1V0Y, [13], and in a controversialβ-phosphoglucomutase structure [14,15] PDB code 1O08 [16]).

A variety of anions have been employed for the visualization of phosphoryl transfer enzymes as transition state mimics or as mimics of phosphorylated states. Metal oxoanions such as molybdate [17] and tungstate [18] have also been employed as phosphate mimics in place of vanadate. Vanadium, with an atomic number of 23, however, possesses a number of electrons that are much closer to phosphorus (atomic number 15) than molybdenum (atomic number 42) or tungsten (atomic number 74). Tungsten is moreover such a heavy element that the resulting electron density can obscure surrounding atoms, making map interpretation difficult, particularly at medium to lower resolution [18]. Furthermore, tungstate has a propensity to form hexacoordinate, octahedral complexes that are not accurate transition state mimics [18,19]. Another advantage of vanadate over other metal oxoanions is the apparent relative ease with which vanadate has been observed to derivatize with other molecules. In one recent case, this property has allowed the self-assembly of a quaternary complex around vanadate that was a mimic for the transition state for cleavage of a phosphodiester substrate, with a histidine, tyrosine and a ribose as ligands [20,21].

Aluminum fluoride, beryllium fluoride and nitrate ion have also been employed in macromolecular crystallography as phosphate analogs [22,23]. The ions AlF_4^-, BeF_3^-, and NO_3^- have a propensity to occupy phosphate-binding sites because they approximate the shape and charge of phosphate. However aluminum fluoride and beryllium fluoride are not isosteric with the trigonal planar PO_3 moiety of a phosphotransfer transition state in a phosphoryl transfer reaction: aluminum fluoride is a square planar ion and the beryllium fluoride ion is non-planar. Nitrate ion is the best steric mimic for the PO_3 moiety, but this compound lacks vanadate's ability to form covalent bonds. In all cases in the PDB to date, these compounds either mimic the γ phosphate during hydrolysis of nucleotide triphosphates, or mimic phosphorylated residues on proteins. Although these compounds have been useful for unraveling catalytic mechanisms, particularly in the case of a series of structures including aluminum fluoride, beryllium fluoride and vanadate with myosin-ADP [22,24], vanadate appears to have several advantages as a transition state mimic.

2. Overview of enzyme–vanadate complexes

Of the 27 112 macromolecular structures in the Protein Data Bank as of September 7, 2004 [25], only 39 include protein-vanadate complexes (see Table 1). This collection of structures includes 23 distinct proteins that encompass 19 unique E.C. numbers. These enzymes represent classes 1, 2, 3 and 5 of the six E.C. groups (categorized as oxidoreductases, transferases, hydrolases and isomerases, respectively). Of these 39 structures, all but six exhibited covalent bonds (defined by bond length) to vanadate and 21 have been classified as representing transition state analogs (although the case of ribonuclease A has been the subject of some debate [6,26,27]). All of the vanadate complexes classified as transition state mimics exhibit trigonal bipyramidal or distorted trigonal bipyramidal geometry around the vanadium, and at least one covalent linkage exists between the vanadium atom and atoms of the enzyme or substrate molecules as will be discussed further below.

The structures classified as not representing transition state mimics fall under one of three categories: Either tetrahedral (presumably VO_4^{3-}) vanadate was bound in a non-covalent fashion [28–31]; or vanadate had formed a cyclic covalent complex with the ribose of a nucleotide that did not represent a possible transition state [32,33]; or the vanadate was present as part of an oligomeric vanadate complex that was incapable of forming a transition state mimic [34,35] (see below for a discussion of vanadate polymerization).

Nine additional structures listed in Table 1 are examples of fungal haloperoxidases, which use a covalently bound vanadate cofactor to catalyze reactions that halogenate organic compounds. The vanadate moiety in the haloperoxidases was not added by the crystallographers, but rather is native to the enzyme. It is interesting to note that phosphatase activity can be catalyzed by apohaloperoxidases, and phosphatases can be converted to haloperoxidases in the presence of vanadate and hydrogen peroxide. Thus, the haloperoxidases can be thought of vanadate-inhibited (i.e., transition state analog-containing) versions of phosphatase enzymes. We will not comment on these cases here further.

3. Technical considerations

Perhaps, the largest technical challenge in working with vanadate is overcoming the compound's tendency to oligomerize to form dimeric and polymeric species in solution at neutral pH [36,37]. The predominant species of vanadate (V) from pH 2 to 6 is a decameric species ($V_{10}O_{28}^{6-}$) that exhibits a bright orange-yellow color. Obviously, this nearly 1 kDa cluster is too large to form an enzyme–vanadate complex capable of providing insight into phosphoryl transfer reactions. At alkaline pH, the monomeric species of vanadate is more prevalent, and many researchers have prepared "activated" solutions of monomeric orthovanadate using a method similar to that described by Gordon [38]. In short, this method involves adjusting the pH of a stock solution of sodium orthovanadate above 10 and heating near 100 °C until the solution is clear and colorless.

Of course, the vast majority of proteins require a pH below 10 for crystallization, so the use of very high pH values for the formation of enzyme–vanadate complexes is impractical. Indeed, the structures of enzymes complexed with monomeric vanadate reported in the Protein Data Bank, excluding the haloperoxidases, which use vanadate as a cofactor, came from crystals that were grown at pH values ranging from 5.0 to 7.8. Given the relatively small number of vanadate complexes in the Protein Data Bank, this pH range should not necessarily be considered limiting. Nevertheless, this pH range covers approximately 50% of the entries in the Biological Macromolecule Crystallization Database [39], which lists crystallization conditions for a subset of entries in the Protein Data Bank. It appears that the use of an "activated"

Table 1
Summary of enzyme–vanadate complexes in the Protein Data Bank

Protein	TS mimic?	# bonds to V	Ligands	pH	Res. (Å)	E.C. number	Enzyme Type	PDB ID	Ref.
Tyrosyl-DNA phosphodiesterase (Tdp1)	Y	3	Nε of His263, OH of tyrosine, O3′ of DNA	7.8	2.3	3.1.4.- (unclassified)	Phosphodiesterase	1NOP	[21]
Tdp1	Y	3	Nε of His 263, O1 and O2 of glycerol	7.8	2.05	3.1.4.-	Phosphodiesterase	1MU9	[18]
Tdp1	Y	3	Nε of His263, OH of tyrosine, O3′ of DNA	7.8	1.70	3.1.4.-	Phosphodiesterase	1RFF	[20]
Tdp1	Y	3	Nε of His263, OH of tyrosine, O3′ of DNA	7.8	2.20	3.1.4.-	Phosphodiesterase	1RFI	[20]
Tdp1	Y	3	Nε of His263, OH of tyrosine, O3′ of DNA	7.8	2.10	3.1.4.-	Phosphodiesterase	1RG1	[20]
Tdp1	Y	3	Nε of His263, OH of tyrosine, O3′ of DNA	7.8	2.10	3.1.4.-	Phosphodiesterase	1RG2	[20]
Tdp1	Y	3	Nε of His263, OH of tyrosine, O3′ of DNA	7.8	2.00	3.1.4.-	Phosphodiesterase	1RGT	[20]
Tdp1	Y	3	Nε of His263, OH of tyrosine, O3′ of DNA	7.8	2.22	3.1.4.-	Phosphodiesterase	1RGU	[20]
Tdp1	Y	3	Nε of His263, OH of tyrosine, O3′ of DNA	7.8	2.30	3.1.4.-	Phosphodiesterase	1RHO	[20]
Scallop Myosin S1	Y	1	β-Phosphate of ADP	6.0	2.6	3.6.4.1	ATPase	1QVI	[42]
Myoe Motor domain	Y	1	β-Phosphate of ADP	7.2	3.0	3.6.4.1	ATPase	1LKX	[44]
Phosphoglucomutase	Y	2	Oγ of Ser116, O6 of Glucose α-1-phosphate-6-vanadate	6.5	2.7	2.7.5.1	Phospho-transferase	1C4G	[45]
Scallop Myosin S1	Y	1	β-Phosphate of ADP	6.0	4.2	3.6.4.1	ATPase	1DFL	[41]
Alkaline phosphatase	Y	1	Oγ Ser102	7.5	1.9	3.1.3.1	Phosphatase	1B8J	[17]
Estrogen sulfotransferase	Y	1	Phosphate of adenosine 3′,5′ diphosphate	6.5	2.1	2.8.2.4	Sulfotransferase	1BO6	[46]
Dictyostelium myosin	Y	1	β-Phosphate of ADP	7.0	1.9	3.6.4.1	ATPase	1VOM	[24]
Ribonuclease A	Y	2	O2′ and O3′ of uridine	6.2	1.3	3.1.27.5	Ribonuclease	1RUV	[26]
Ribonuclease A	Y	2	O2′ and O3′ of uridine	6.2	1.3	3.1.27.5	Ribonuclease	6RSA	[26]
Rat acid phosphatase	Y	1	Nε of His12 (nucleophile in enzyme)	5.4	3.0	3.1.3.2	Phosphatase	1RPT	[47]
Hairpin ribozyme	Y	3	02′ and 03′ of G +1, +O5′ of A-1	N.R	2.2	3.1.27. -	Ribonuclease (ribozyme)	1M5O	[40]
PhoE	Y	1+	Trivanadate bound: One to Nε of His10 (nucleophile), different vanadate to Glu83.	5.0	2.0	5.4.2.1		1H2F	[48]
KIF1A Kinesin	Y	1	β-Phosphate of ADP	7.0	2.24	3.6.4.4	ATPase	1VFZ	[53]
Cyclic nucleotide phosphodiesterase	N	2	O2′ and O3′ of uridine Substrate mimic	5.0	2.4	3.1.4.17	Phosphodi-esterase	1JH7	[32]
APS kinase	N	2+	O2′, O3′ of ribose of ADP, plus 3′OH of symmetry-related ADP	6.5	1.43	2.7.1.25	Phospho-transferase	1M7G	[33]
SurE protein (phosphatase)	N	0	Tetrahedral vanadate bound non-covalently	7.5	1.9	3.1.3.-	Phosphatase	1J9L	[28]
Human cyclin dependent kinase	N	0	Partially disordered meta-vanadate ($V_{10}O_{28}$) bound non-covalently	7.4	2.9	2.7.1.37	Kinase	1DKT	[35]
Ribonuclease T1	N	0	Tetrahedral vanadate bound non-covalently	5.0	1.8	3.1.27.3	Ribonuclease	3RNT	[29]
Phosphoglycerate mutase	N	0	Tetravanadate bound non-covalently	8.75	1.3	5.4.2.1	Mutase	1E59	[30]
Bacterial abc transporter	N	0	n-cyclo-tetrameta-vanadate bound non-covalently	8.0	3.2	3.6.3.33	ATPase	1L7V	[34]

Table 1 (*continued*)

Protein	TS mimic?	# bonds to V	Ligands	pH	Res. (Å)	E.C. number	Enzyme Type	PDB ID	Ref.
C3 exoenzyme from *C. botulinum*	N	0	Tetrahedral vanadate and n-cyclo-tetrametavanadate bound non-covalently	5.5	1.89	2.4.2.30	NAD(+) ADP-ribosyltransferase	1UZI	[31]
Haloperoxidase	N	1	Nε of His486	8.3	2.05	1.11.1.10	Haloperoxidase	1QI9	[49]
Chloroperoxidase	N	1	Nε of His496	8.0	2.1	1.11.1.10	Chloroperoxidase	1VNC	[50]
Chloroperoxidase	N	1	Nε of His496	8.0	2.03	1.11.1.10	Chloroperoxidase	1IDQ	[51]
Chloroperoxidase	N	1	Nε of His496	8.0	2.24	1.11.1.10	Chloroperoxidase	1IDU	[51]
Chloroperoxidase (R360A)	N	1	Nε of His496	8.0	2.35	1.11.1.10	Chloroperoxidase	1VNF	[52]
Chloroperoxidase (D292A)	N	1	Nε of His496	8.0	2.15	1.11.1.10	Chloroperoxidase	1VNE	[52]
Chloroperoxidase (H404A)	N	1	Nε of His496	8.0	2.2	1.11.1.10	Chloroperoxidase	1VNG	[52]
Chloroperoxidase (H496A)	N	1	Nε of His496	8.0	2.11	1.11.1.10	Chloroperoxidase	1VNH	[52]
Chloroperoxidase	N	1	Nε of His496	8.0	2.11	1.11.1.10	Chloroperoxidase	1VNI	[52]

Summary of protein-vanadate complexes in the Protein Data Bank. The "TS mimic?" identifies whether or not the authors of the original citation have identified the crystal structure as a transition state mimic. "#bonds to V" records the number of covalent bonds between vanadate and other (non-water oxygen) ligands, which are listed in the "ligands" column. The pH column lists the reported pH for crystallization of the complex. Resolution is the resolution of the published crystal structure. The E.C. number/enzyme type columns record the E.C. classification number and common name of the enzyme, respectively. In cases where enzymes have not been assigned an E.C. number, the fourth position is marked with a dash. PDB ID lists the accession number in the RCSB Protein Data Bank (www.rcsb.org/pdb).

(i.e., pH > 10) vanadate stock solution can be effective in producing complexes with protein solutions buffered near neutral pH (as in [21,24,40]) because monomeric vanadate may be able to form an enzyme–vanadate complex before a prohibitive amount of polymerization takes place. However, there have been instances where very alkaline solutions of vanadate have not been necessary to achieve crystal structures of vanadate complexes, such as the case of alkaline phosphatase ([17] see below).

4. Insights from specific vanadate complex structures

The ability to visualize a transition state mimic is a powerful tool for understanding enzyme mechanism. Structures of transition state analogs can clarify the role of active site amino acid residues and reveal conformational changes that may take place in the course of the catalytic cycle. Enzyme mechanisms sometimes involve dynamic rearrangements of active site residue conformations and/or domain movements. An ensemble of structures representing different points along a reaction pathway is required for a complete understanding of an enzyme's mechanism. For enzymes that catalyze chemistry on a phosphate group, enzyme–vanadate complexes have provided crucial insights into the mechanisms of the transferase APS kinase; hydrolases including ribonuclease T1, the SurE phosphatase, cyclic nucleotide phosphodiesterase, acid phosphatase, alkaline phosphatase, myosin, tyrosyl-DNA phosphodiesterase and the hairpin ribozyme; the isomerases phosphoglycerate mutase and PhoE; and, as a naturally occurring cofactor, the oxidoreductases chloroperoxidase and haloperoxidase (see Table 1). The four detailed examples that follow have provided valuable insights into enzyme mechanism, and collectively demonstrate the ability of vanadate to mimic transition states for enzymes involving monoester substrates (which are generally presumed to follow a "dissociative" mechanism) as well as diester substrates (presumed to have more "associative" mechanisms).

The vanadate complex with *E. coli* alkaline phosphatase [17] was obtained by soaking existing crystals of the enzyme in a stabilization solution at pH 7.5 that included 100 μM NH_4VO_3 from a stock solution prepared at a pH of 8.0. Even though the vanadate solution was not "activated" to ensure the predominance of monomeric vanadate, vanadate was observed in the active site of the resulting crystal structure. The vanadate moiety adopted trigonal bipyramidal geometry, with the Oγ of Ser102 contributing one apical ligand (Fig. 1a). This structure was published as part of a three-structure series (PDB entries 1HJK, 1ALK and 1B8J) that also included the enzyme non-covalently bound to inorganic phosphate and a phosphorylated mutant enzyme. Taken as a whole, these structures provide strong support for the role of a bound Zn ion in activating a water molecule for attack on the phosphoseryl intermediate. Furthermore, the three structures allow the trajectory of the nucleophilic water molecule during the hydrolysis of the phosphoseryl intermediate to be traced. In a structure of a phosphorylated alkaline phosphatase H331Q mutant (PDB ID 1HJK), a water molecule was observed coordinated to Zn_1 at a distance of 2.2 Å from the Zn and 3.4 Å from the phosphate. In the vanadate complex, this "activated" water molecule is observed as the free oxygen bound at an apical position of vanadate, 2.4 Å from Zn_1 and 1.9 Å from the vanadium atom.

Vanadate complexes with the ATPase myosin [24,41,42] also exhibit trigonal bipyramidal geometry around the vanadium atom, but there are no covalent linkages directly to the enzyme, only one apical ligand to the β-phosphate of ADP is formed (Fig. 1b). These structures represent a transition state mimic for the hydrolysis of the γ-phosphate from ATP. This ADP-vanadate structure provided a more realistic transition

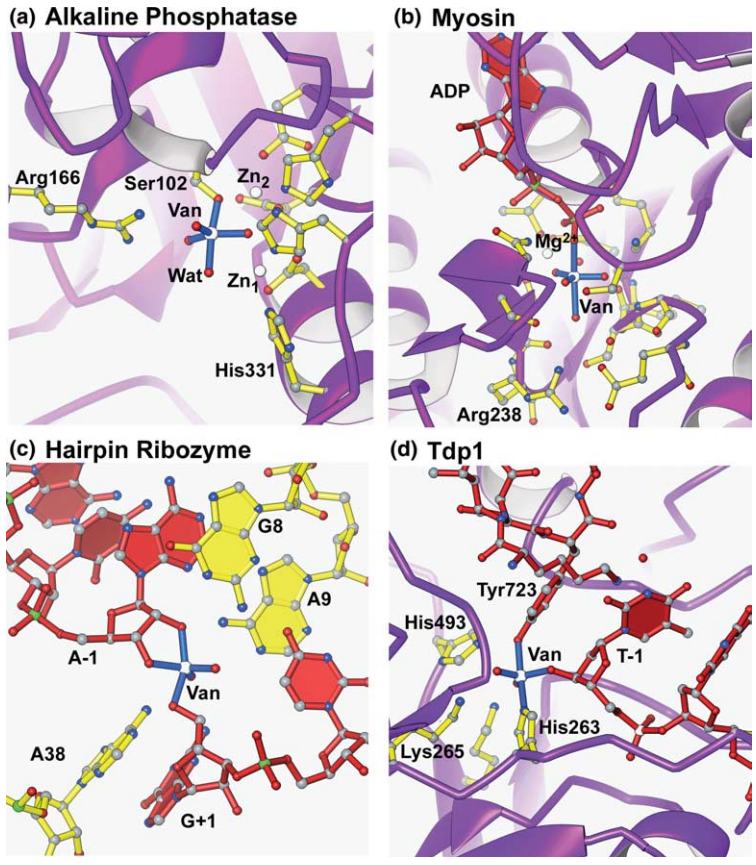

Fig. 1. Selected enzyme–vanadate complexes. (a) Alkaline phosphatase–vanadate complex from PDB ID 1B8J [17]. The protein structure is displayed as a purple ribbon model with amino acid residues displayed as ball-and-stick structures with yellow bonds. The vanadate moiety (Van) is also displayed as a 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 [24]. 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 [40]. 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 [21]. 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.

state mimic than a previously solved structure with ADP and aluminum fluoride, where the AlF_4 moiety occupied the γ-phosphate binding pocket but adopted a square planar arrangement [24]. The vanadate–ADP complex was trapped in the active site of myosin prior to crystallization by addition of 2 mM ADP, 3 mM "activated" sodium orthovanadate and 1 mM $MgCl_2$ [24]. The resulting 1.9 Å structure of ADP-vanadate in a truncated *Dictyostelium* myosin revealed how the protein ligands and water structure surrounding the γ-phosphate pocket were oriented to stabilize a water molecule in an appropriate position for in-line nucleophilic attack on the γ-phosphorus of ATP [24]. Beyond the active site of myosin, the vanadate–ADP structures also captured an important snapshot of conformational changes that play a role in the transduction of the chemical energy of hydrolysis of ATP into mechanical movement.

The ability of vanadate to readily derivatize and accept many different ligands was demonstrated in a stabilized transition state mimic of the hairpin ribozyme, where a vanadate in the active site formed three covalent bonds with surrounding ribonucleotides (Fig. 1c). This complex was obtained by a slight modification of known crystallization conditions with an addition of an alkaline (above pH 10) stock solution of NH_4VO_3 (Adrian Ferre-D'Amare, personal communication, and [40]). In the structure, one apical and one equatorial ligand were contributed by the 2′ and 3′ oxygens of one ribonucleotide and represented the attacking nucleophile and the non-cleaved bridging oxygen, respectively. The other apical ligand was contributed by the 5′ oxygen of a second ribonucleotide and represented the bridging oxygen of the phosphodiester to be cleaved. The resulting pentacoordinate geometry of the vanadate moiety is that of a distorted trigonal bipyramid and is described by the authors as "an approximate mimic of the transition state that is otherwise impossible to visualize experimentally" [40]. Comparison of the vanadate complex with precursor and product structures revealed that the hairpin ribozyme has apparently evolved to maximize the hydrogen bonding interactions with the trigonal bipyramidal transition state, and showed that the active site was essentially rigid, with molecular motions during phosphodiester cleavage confined to

the scissile phosphate itself and the ribose moiety that contains the attacking nucleophile. These observations led to the proposal that a common mechanism in RNA enzymes involves transition state stabilization coupled with precise positioning of substrate [40].

The versatility and power of enzyme–vanadate complexes has also been demonstrated with a series of quaternary complexes around vanadate in the active site of the DNA repair enzyme human tyrosyl-DNA phosphodiesterase (Tdp1). Tdp1 catalyzes the removal of tyrosine from a 3′ phosphate at the end of a strand of DNA, a substrate that occurs as a stalled intermediate in defective topoisomerase I–DNA complexes. A transition state mimic for cleavage of this substrate was visualized in a crystal structure where a trigonal bipyramidal vanadate moiety had apical ligands composed of an active site histidine side chain and a peptide tyrosine hydroxyl representing the attacking nucleophile and the leaving group, respectively, and an apical ligand composed of the 3′ hydroxyl of a single-stranded DNA oligonucleotide representing the non-cleaved portion of the phosphodiester [21] (Fig. 1d). This structure was created by co-crystallization of a mixture of 5.5 mg/ml Tdp1, 3 mM ''activated'' sodium orthovanadate from a 100 mM stock solution, 20 nmol/μl of a topoisomerase-derived octapeptide, and 5 nmol/μl of a single-stranded hexanucleotide. The transition state mimic apparently self-assembled on the enzyme during crystallization. This quaternary complex answered many questions about the mechanism of Tdp1. First of all, the binding of vanadate to His263 resolved a question about which of the two histidine residues in the active site served as the nucleophile in the initial cleavage step. The structure also confirmed the substrate binding mode and the observed conformation of the substrate mimic provided strong evidence that the topoisomerase I–DNA complex must necessarily undergo some form of degradation prior to cleavage by Tdp1 [21]. Follow-up studies with the Tdp1 system have revealed that quaternary complexes around vanadate can still be formed when the DNA and peptide sequences are changed, and that the peptide moiety can be replaced by non-peptide ligands [20]. The modular nature of vanadate ligands in the active site has led to interest in the use of vanadate complexes as a tool for the development of structure-based inhibitors [20].

5. Perspectives

Vanadate is a powerful tool for the formation of approximate transition state analogs for enzymes that perform chemistry on phosphate. The visualization of such structures is essential for understanding the dynamics of enzyme mechanisms, and can often provide key insights into enzyme structure and function. A survey of the Protein Data Bank has revealed that structures of vanadate complexes have been solved for a diverse subset of enzymes, encompassing four of the six E.C. categories. The crystallization conditions used to achieve these structures have likewise been diverse, ranging in pH from 5.0 to 7.8. Furthermore, vanadate has been successful in forming pentacoordiante transition state mimics for enzymes that cleave phosphate monoesters (generally thought to follow a ''dissociative'' mechanism) as well as enzymes that cleave phosphate diesters (generally thought to follow a more associative mechanism). Given the versatility of vanadate and the apparent ease with which many of these complexes were obtained, it is somewhat surprising that this method is not used more frequently. The number of vanadate structures appears to fall far short of the potential – for example, although there are over 300 entries in the Protein Data Bank for various phosphatases, only two phosphatase–vanadate structures are deposited (a third structure, for a phosphotyrosyl phosphatase has been solved but is not currently in the PDB [43]). Given the apparent versatility of vanadate and the first-hand experience of these authors, we propose that the use of vanadate to form transition state mimics for phosphoryl transfer enzymes is a component of the macromolecular crystallographer's toolkit that could be brought to bear with greater frequency and efficacy.

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