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MINI-REVIEW

Structure and function of vanadium compounds in living organisms

Dieter Rehder

Institut für Anorganische und Angewandte Chemie, Universität Hamburg, Hamburg, Germany

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Vanadium has been recognized as a metal of biological importance only recently. In this mini-review, its main functions uncovered during the past few years are addressed. These encompass (i) the regulation of phosphate metabolizing enzymes (which is exemplified for the inhibition of ribonucleases by vanadate), (ii) the halogenation of organic compounds by vanadate-dependent non-heme peroxidases from seaweeds, (iii) the reductive protonation of nitrogen (nitrogen fixation) by alternative, i.e. vanadium-containing, nitrogenases from N_2-fixing bacteria, (iv) vanadium sequestering by sea squirts (ascidians), and (v) amavadine, a low molecular weight complex of V(IV) accumulated in the fly agaric and related toadstools. The function of vanadium, while still illusive in ascidians and toadstools, begins to be understood in vanadium–enzyme interaction. Investigations into the structure and function of model compounds play an increasingly important role in elucidating the biological significance of vanadium.

Keywords: vanadium biochemistry, ribonuclease inhibition, bromoperoxidase, vanadium nitrogenase, amavadine, vanadium in ascidians

Introduction

Vanadium is usually introduced to students as a metal capable of beautiful color changes when passing through its various oxidation states. Panchromium, the name given to it by its initial discoverer in 1813, the Spanish mineralogist del Rio, referred to this ability. Del Rio withdrew his discovery, but the history of vanadium remained colorful. It was rediscovered by the Swedish chemist Sefström in 1831 and named vanadium after Vanadis, a nickname of the Germanic Goodess Freya (or Frija), meaning 'beauty' (for a review of the discovery of vanadium, see Hoppe *et al.* 1990).

Vanadium belongs to those intriguing metals, the biological relevance of which, while recognized already at the beginning of our century, has become generally established only in the last few years (for recent reviews, see Chasteen 1990, Rehder 1991).

Address for correspondence: D. Rehder, Institut für Anorganische und Angewandte Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, W-2000 Hamburg 13, Germany.

Like molybdenum, vanadium is available in anionic and cationic forms, the most common ones being, under physiological conditions, vanadate ($H_2VO_4^-$) and vanadyl (VO^{2+}). The standard potential for the pair $H_2VO_4^- + 4H^+ + e \rightleftharpoons VO^{2+} + 3H_2O$ is 1.31 V. Hence, vanadyl undergoes autoxidation to vanadate in the presence of oxygen, and vanadate in turn is reduced by reductants such as glutathion, ascorbate and NADH. This facile change between V(V) and V(IV) is an especially interesting feature: on the one hand, vanadium can act as a competitor to phosphate (HPO_4^{2-}); on the other hand, it acts as a transition metal ion which competes with other metal ions in coordination to biogenic compounds. There are a few other forms of vanadium that may be present in aqueous media at physiological pH, and these are anionic or cationic again. Cationic forms are VO^{3+}, VO_2^+, V(IV) and V(III), which can exist at pH values around 7 stabilized by complex formation only (this is also true for VO^{2+}). Among the anions are divanadate ($H_2VO_4^{2-}$) and tetravanadate ($V_4O_{12}^{4-}$; Crans & Schelble 1990). Since the minimum concentrations for their

existence are $c(V) > 0.1$ mM (the average $c(V)$ in human tissue amounts to 0.1–1 μM) oligovanadates are important at toxic levels of vanadate only, or within special cell compartments where vanadate is accumulated.

The biological impetus of vanadium known to date may be summarized under the following five headings (all the references given have review character):

(1) Regulation of phosphate-metabolizing enzymes (Gresser & Tracey 1990). This is a general function and makes vanadium a trace metal possibly essential for all organisms. The active species is vanadate, the basis of its function is the vanadate–phosphate antagonism. The *in vivo* reduction of vanadate to VO^{2+} has implications for this regulatory role. Medical implications, such as the insulin mimetic effects of vanadate and vanadyl, come in.

(2) Accumulation in sea squirts—ascidians (Wever & Kustin 1990). Here, vanadium is present in specialized vacuoles in the ascidian blood stream in the form (of complexes) of V(IV) or V(III). Their nature is still under debate, as is the function of vanadium.

(3) Accumulation in the fly agaric toadstool (Fraústo da Silva 1989), which contains a low molecular weight V(IV) compound (amavadine) with an unknown function.

(4) Catalysis of the halogenation of organic substrates by vanadate-dependent haloperoxidases from seaweeds (Butler & Carrano 1991, Vilter 1991, Wever & Kustin 1990). The active forms of these non-heme enzymes contain V(V); the coordination environment is under debate.

(5) Catalysis of the reductive protonation of N_2 to NH_4^+ by vanadium nitrogenases from nitrogen-fixing bacteria such as *Azotobacter* (Eady 1988). Vanadium, probably in the oxidation states V(II)/V(III), is part of an ion–vanadium–sulfer cluster.

I will briefly address all five topics, emphasizing structural aspects including those of model compounds.

Vanadates as phosphate analogs and the interaction of vanadium with proteins

Three cases may be distinguished: (i) the inhibition of enzymatic phosphorylation reactions by vanadate; (ii) the recognition by proteins of vanadate, vanadate esters and vanadate anhydrides as alternate (to phospate) ligands/substrates; and (iii) the alternate (to other metals) binding of vanadate and vanadyl to apo-metallo-enzymes with various functions. The third case has widely been exploited to characterize metal binding sites by EPR and ^{51}V-NMR. Recent examples are EPR/ENDOR studies on VO^{2+} substituted xylose isomerase (Bogumil *et al.* 1991), and the investigation of the vanadium transport by transferrin (Tf) on the basis of EPR (Chasteen *et al.* 1986) and ^{51}V-NMR (Butler & Eckert 1989). The latter study has led, in connection with other spectral data, to a formulation of the binding sites for vanadate in Tf shown in Figure 1(**1**).

While binding of vanadate and vanadate derivatives as ligands alternate to phosphate may result in a stimulation of enzymatic activity (Stankiewicz *et*

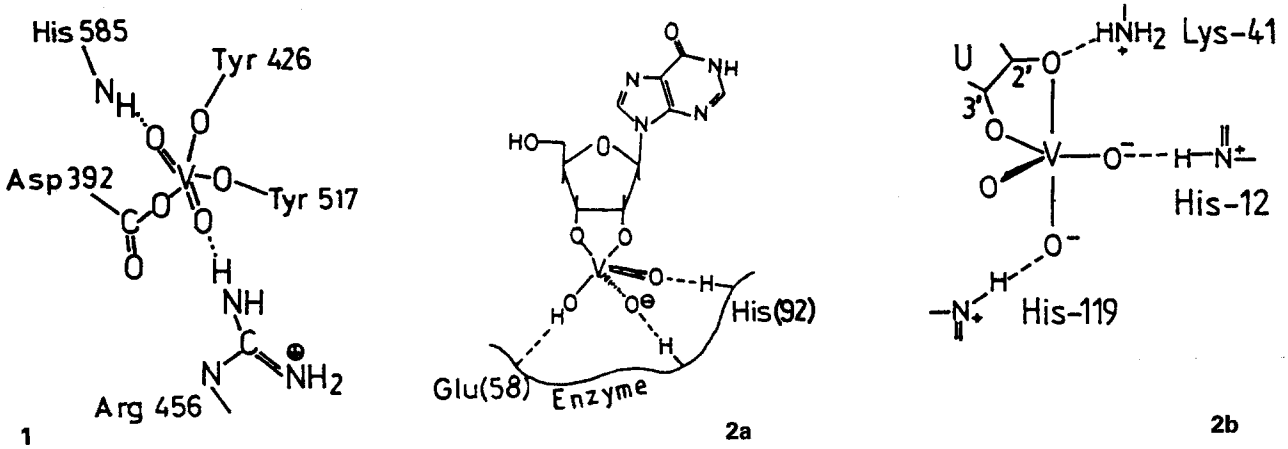


Figure 1. Proposed coordination spheres for: (**1**) the vanadate–transferrin complex (Butler & Carrano 1991); and (**2**) the ternary complexes formed between vanadate, inosine and (**a**) ribonuclease T_1 from *Aspergillus oryzae* (Rehder *et al.* 1989a, Richter & Rehder 1991) and (**b**) ribonuclease A from bovine pancreas (Borah *et al.* 1985). Structure (**1**) contains the *cis*-VO_2^+ moiety.

al. 1987, Mendz *et al.* 1990), phosphate metabolizing enzymes are usually inhibited by even very low concentrations of vanadate. This has been shown for Na,K-ATPase by Cantley & Josephson as early as 1977 (Cantley *et al.* 1977), and documented since then for a large number of phosphorylation and phosphoryl transfer enzymes (Gresser & Tracey 1990). Examples that have been studied comprehensively are phosphoglucomutase (Percival *et al.* 1990, Ray *et al.* 1990) and ribonucleases. In these cases, vanadium, which (in contrast to phosphorous) can form stable complexes with coordination numbers 5–8 by virtue of its low-lying 3d orbitals, is believed to act as the center of a stable transition state analog (Figure 1; **2**), thus blocking off enzymatic action.

The interaction between vanadate, enzymes and enzyme substrates has stimulated investigations into the structure, kinetics and mechanistics of small binary and/or ternary model compounds. Simple compounds are commonly used to model binding of vanadate to side-chain and/or back-bone functionalities of enzymes and their substrates. Proposed structures for complexes formed between vanadate and biogenic or biomimetic ligands in connection with a better understanding of the inhibitory action of vanadate towards ribonucleases are shown in Figure 2. The pentavalent vanadium complex (**3**) exhibits a trigonal-bipyramidal geometry and hence may be considered a transition state analog (as depicted in Figure 1; **2**) for the cleavage of the phosphoester bond in RNAses. Complexation between vanadate and nucleosides (such as inosine) in aqueous solution has been demonstrated to proceed via a pentavalent monomer (Figure 2; **4**) with a complex formation constant of $2\ \text{M}^{-1}$ (Tracey & Leon-Lai 1991) to a dimeric 2:2 complex with a dimerization constant of $\sim 6 \times 10^6\ \text{M}^{-1}$ (Tracey *et al.* 1990, Richter & Rehder 1991), possibly of structure **5** or **6**, i.e. with a coordination number 5 or 6 for vanadium. Binary complexes between vanadate and peptides (proteins) may be formed by hydrogen bonding (**8**) or direct coordination (**7**).

Enzymes with vanadium in the active center

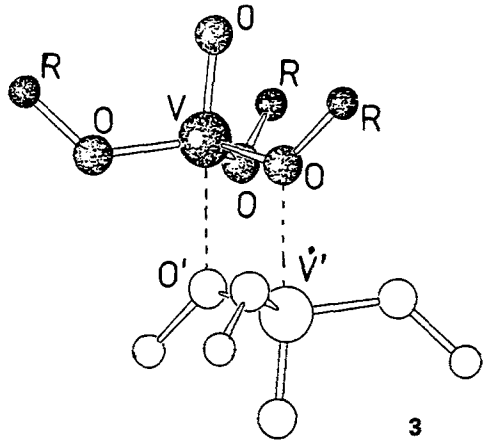
Vanadate-dependent haloperoxidases

These remarkable enzymes were discovered by Vilter (Vilter 1984) in the cell wall of the marine alga knotted wrack (*Ascophyllum nodosum*) and have since been isolated in various brown and red algae and in a lichen (Wever 1988, Wever & Kustin 1990). The molecular weight of the main isoenzyme from *A. nodosum*, A.n.1, amounts to 100 kDa (by sedimentation). There is no apparent subunit structure. The active form of the enzyme contains V(V). It can be desactivated by reduction (to VO^{2+}) with dithionite and by dialysis against EDTA/citrate. The reconstitution with vanadate is a slow process, taking about 24 h until full activity is regained and suggesting a shuttle mechanism (Figure 3) for vanadate incorporation (Rehder *et al.* 1991). The holoenzyme in fact contains two V per molecule (Vilter & Rehder 1987), possibly distributed in a 1:1 ratio between active site ($K = 3 \times 10^7\ \text{M}^{-1}$) and surface vanadate ($K = 16\ \text{M}^{-1}$). The spectral properties for the low-affinity binding site on the surface are very similar to those of vanadate–peptide model complexes (Figure 1; **7**). Phosphate, arsenate and molybdate are effective inhibitors. The processes catalyzed by vanadate-dependent peroxidases are formulated in equation (1):

$$\text{Hal}^- + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{HOHal} + \text{H}_2\text{O} \begin{cases} \rightarrow \text{RHal} + \text{H}_2\text{O} \\ \quad + \text{RH} + \text{H}^+ \\ \rightarrow \text{O}_2(^1\Delta_g) + \text{Hal}^- + \text{H}_2\text{O} \\ \quad + \text{H}^+ \end{cases} \quad (1)$$

Hal^- usually is bromide or iodide, but chlorination has also been reported (Soedjak & Butler 1990a). Organic substrates include cytosine, which is brominated to 5-bromocytosine (Soedjak & Butler 1990b). Without substrate, the intermediate hypohalous acid forms singlet oxygen (Everett *et al.* 1990. An interesting point has been addressed by Wever *et al.* (1991), regarding the impact of volatile brominated compounds such as $CHBr_3$ on the biosphere. An estimated 10^4 t per year of $CHBr_3$, produced by A.n.1 and related enzymes from other algae, may well contribute to the overall ozone balance.

There are several proposals, **12–14** depicted in Figure 4, for the structure of the active center, based on EXAFS, EPR/ESEEM, XANES and ^{51}V-NMR. The common feature of all of these preliminary formulations is a coordination number of at least six, and a coordination sphere predominantly occupied by oxygen, including one V=O double bond. The seven-coordinated carboxylato complex **15** in Figure 4 has been proposed as an active site model (Rehder *et al.* 1989b).



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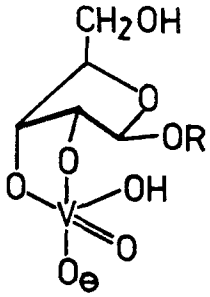

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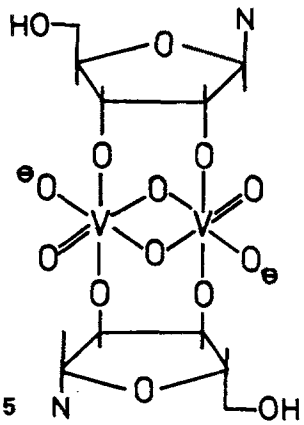

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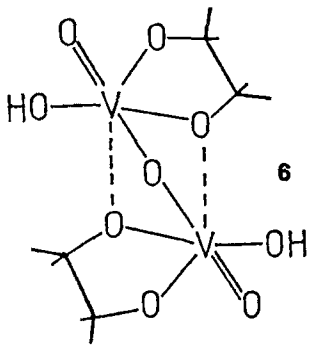

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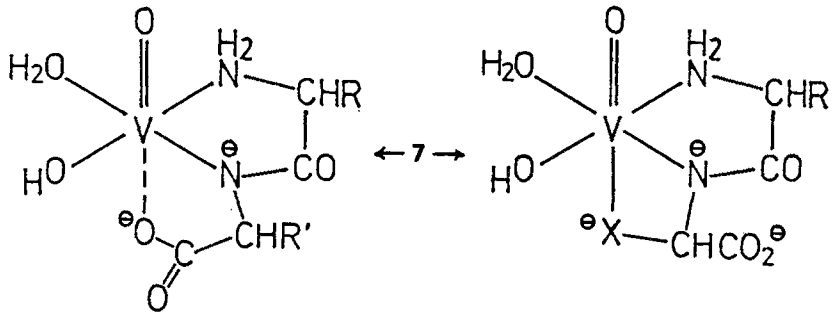

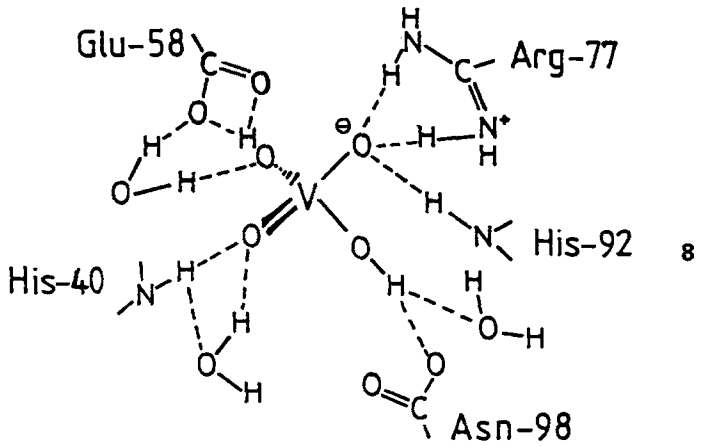

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Figure 2. Model compounds proposed for the interaction between vanadate, enzymes (RNAses) and substrates to RNAses. Compounds (**3**) and (**8**) have been structurally characterized by X-ray analysis; other formulations are based on spectroscopic evidence. (**3**) (R = cyclopentyl): Hillerns *et al.* (1992); (**4**) Geraldes & Castro (1989); (**5**) Crans *et al.* (1991); (**6**) Richter & Rehder (1991), Tracey & Leon-Lai (1991); (**7**) Rehder (1988), Jaswal & Tracey (1991); (**8**) Kostrewa *et al.* (1989). The dashed lines in (**3**) and (**6**) indicate relatively weak bonding interaction.

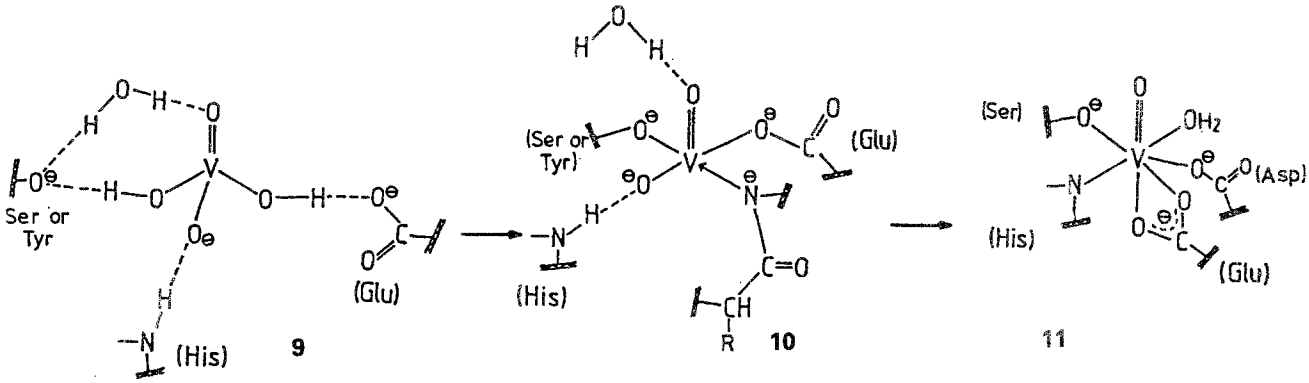


Figure 3. 'Shuttle mechanism' proposed for the stepwise incorporation of vanadate into the active site of A.n.l. The first step (**9**) is hydrogen binding of vanadate to the protein, followed by (**10**) coordination through side-chain function and/or the backbone of the protein surface. For the active site itself (**11**), cf. the formulations shown in Figure 4.

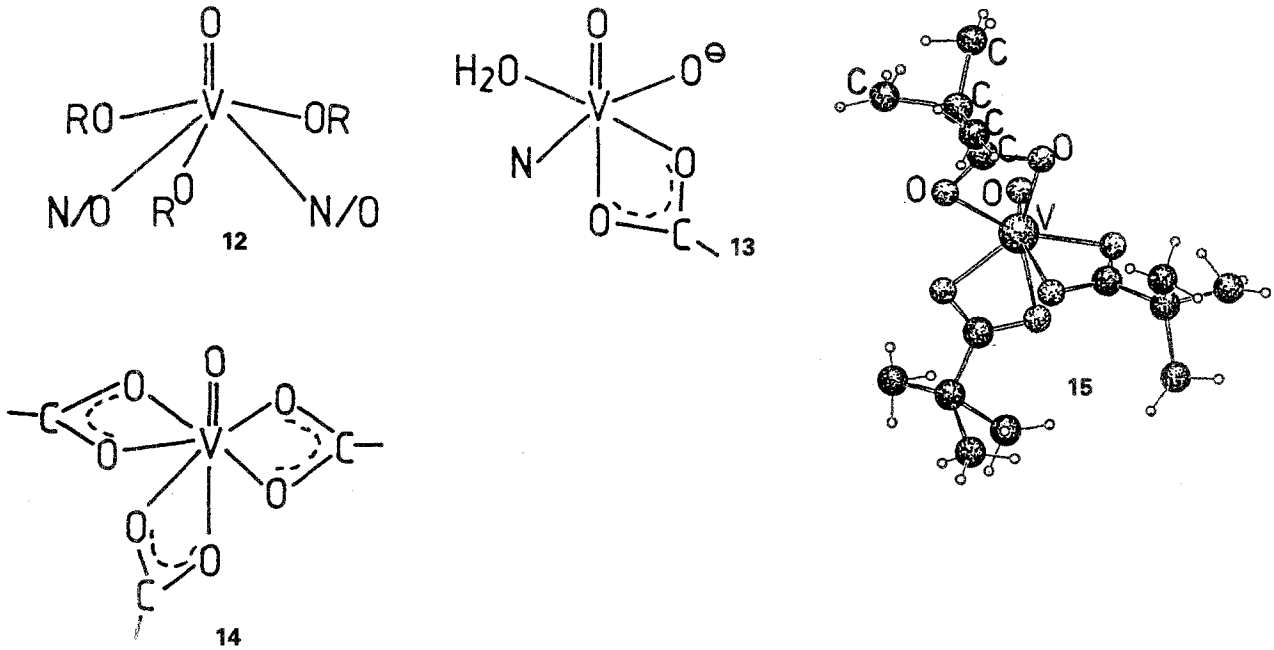


Figure 4. Tentative formulations for the active site in A.n.l. based on (**12**) EXAFS (Arber *et al.* 1989), (**13**) EPR/ESEEM (de Boer *et al.* 1988) and (**14**) XANES (Hormes *et al.* 1988) and ^{51}V-NMR (Vilter & Rehder 1987); (**15**) has been proposed as a model compound for (**14**).

Vanadium nitrogenases

The 'alternative', i.e. vanadium-containing, nitrogenase (V-Nase) is expressed along with molybdenum nitrogenase (Mo-Nase) and iron-only nitrogenase in nitrogen-fixing bacteria belonging to genera such as *Azotobacter, Klebsiella* and *Anabaena*. Those from *A. chroococcum* and *A. vinelandii* were the first to be discovered (Hales *et al.* 1986), and have been thoroughly investigated since (Eady 1988, Miller & Eady 1988). Like Mo-Nase (encoded by the genes *nif*HDK), V-Nase (encoded by *vnf*HDGK) contains two iron proteins and two iron–vanadium proteins (M = 240 kDa for each FeV protein), with an $\alpha_2\beta_2\delta_2$ subunit structure compared with the $\alpha_2\beta_2$ structure of the FeMo protein. The FeV cofactor is similar to the FeMo cofactor regarding both its overall structure and function (equation 2). The metal–sulfur clusters suggested for the FeV cofactor on the basis of Fe K-edge EXAFS (Harvey *et al.* 1990), shown in Figure 5 (**16** and **17**) have to take

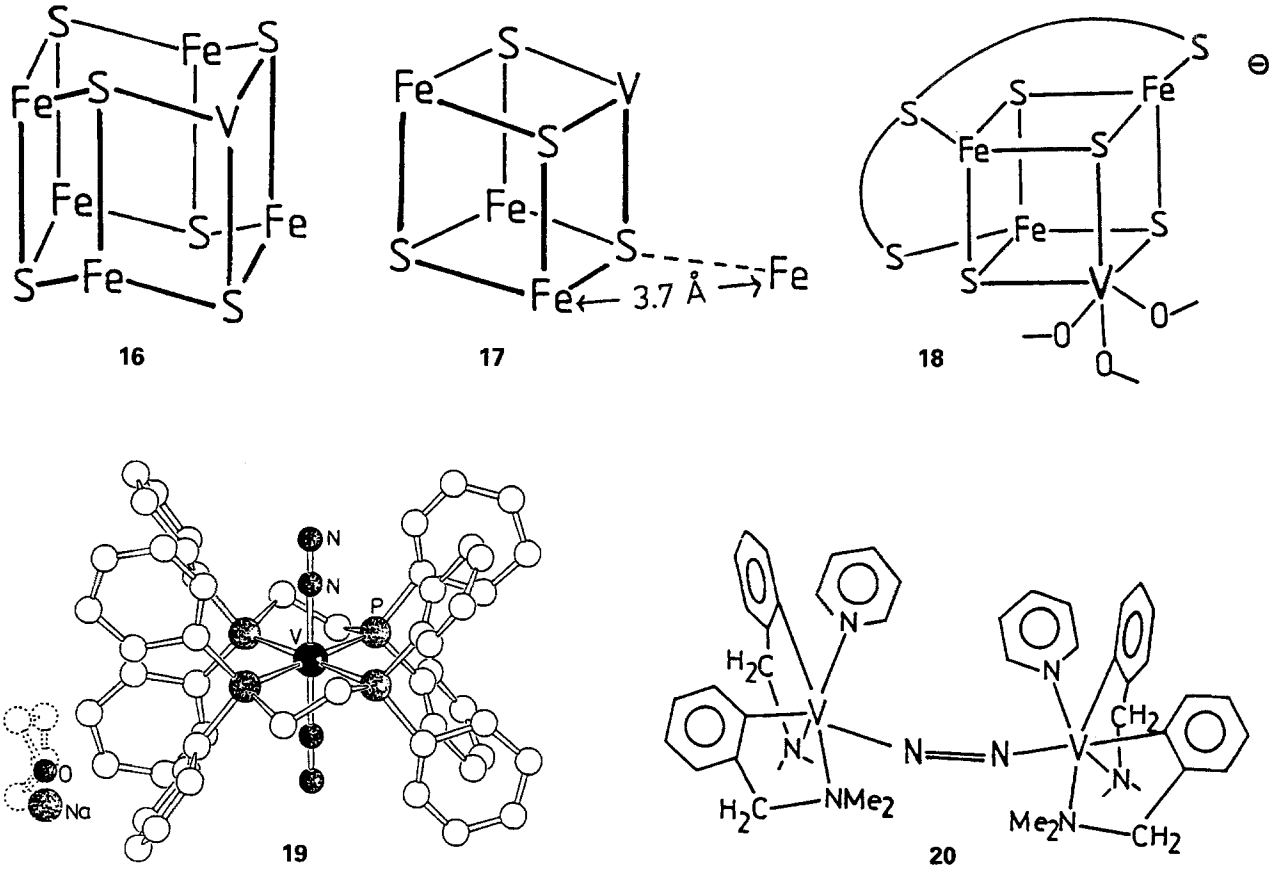


Figure 5. Models for the active site of vanadium nitrogenase: (**16**) and (**17**) are based on EXAFS data (3 d(Fe–S) = 2.24, 2 d(Fe–Fe) = 2.65, 1 d(Fe–V) = 2.75 to 2.90, 1 d(Fe–Fe) = 3.69 Å). (**18**) is a structural model (Ciurli & Holm 1989). The Fe_3VS_4 core is inserted into a semi-rigid trithiophenolate cavitand ligand, modelling the protein matrix. Three of the vanadium coordination sites are occupied by *O* donors. (**19**) (Woitha & Rehder 1990; Rehder *et al.* 1992) and **20** (Edema *et al.* 1989, Leigh *et al.* 1991) are functional models. The ancillary ligand in (**19**) is $Ph_2PCH_2CH_2PPh_2$ (dppe).

into account a long-range order, i.e. an iron–iron distance d(Fe–Fe) of 3.69 Å. The mixed-metal cubane cluster **18** in Figure 5 is a close *structural* model, but lacks the ability to produce ammonia from nitrogen:

$$N_2 + 12e^- + 14H^+ \rightarrow 2NH_4^+ + 3H_2 \qquad (2)$$

(2MgATP are hydrolyzed to MgADP and P_i per e^- transfered).

There are also distinct differences in the functions of V-Nase and Mo-Nase, suggesting a modulation of the final, proton-coupled electron transfer step by the heterometal: (i) V-Nase uses 50% of the electron flux in the production of hydrogen from protons (Mo-Nase: 25%); (ii) small amounts of hydrazine are produced during turn-over by V-Nase as compared with none by Mo-Nase (Dilworth & Eady 1991); (iii) V-Nase reduces acetylene partly to ethane, while, with Mo-Nase, only ethylene evolves. *Functional* models that have been proposed are the V(–I) complex $[Na(thf)][V(N_2)_2(dppe)_2]$ (**19**) and the binuclear V(II) complex **20** (Figure 5). Both complexes yield ammonia in the presence of acids according to equations (3) and (4).

$$[V(N_2)_2]^- + 3H^+ \rightarrow NH_3\,(+ \sim 1\%\ N_2H_4) + 3/2N_2 + V(II) \qquad (3)$$

$$2V(II) + N_2 + 2H^+ \rightarrow 2/3NH_3 + 2/3N_2 + 2V(III) \qquad (4)$$

Recent evidence for the native enzyme, suggesting as 70 Å separation between the two FeV clusters, is not in favor with a bridging N_2 as in **20**.

Accumulation of vanadium

Vanadium in ascidians

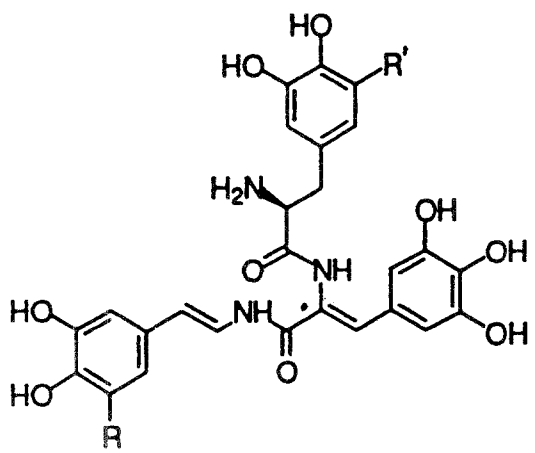
Several species among the ascidians (sea squirts; a primitive vertebrate order belonging to the tunicates) accumulate vanadium to 10^7 times the level in

sea water (where it exists in the form of $H_2VO_4^-$). The vanadium is concentrated up to 0.15 M in specialized blood cells (vanadocytes), termed morula, compartment and signet-ring cells according to their morphological appearance. Depending on the ascidian suborder, vanadium is present mainly in the +III (Phleobranchia) or +IV states (Aplousobranchia) (Brand *et al.* 1989). Its function, originally (but erroneously) thought to be that of an oxygen carrier ('hämovanadin'; Henze 1911), is still illusive, as is the question of the pH within the cell compartments containing vanadium. Hodgson and co-workers, based on an EPR method, favor a low pH of about 1.8 (Frank *et al.* 1988), while Kustin and co-workers (Lee *et al.* 1990) gave a pH of 7.1(2) and 5.0(2) for the cytoplasm and the vacuoles, respectively, of the morula cells of *Ascidia ceratodes*. These data are based on distribution experiments with ^{14}C-labeled organic bases and acids. The controversies regarding the correct pH have been outlined by Smith (1989).

If, in fact, the medium is close to neutral, the question arises of how vanadium is stabilized (V(III) and V(IV) as such cannot exist above pH 3). A VO^{2+}-binding substance containing a reducing sugar and termed *vanadobin* has been isolated by Michibata (1989). Vanadobin may act as an agent reducing vanadate to V(IV) and stabilizing it by coordination. Ascidians contain dark pigments (*tunichromes*; Figure 6; **21**), which are oligopeptides consisting of hydroxy-DOPA units. These pigments are present in the vanadocytes *and* the tunic (where they probably serve as cross-links for the cellulose-like fibrous material which constitutes the tunic). Tunichromes have been shown to be powerful reducing and complexing agents for vanadium (Oltz *et al.* 1988, 1989). Based on these findings, it has become very popular presently to trace back the function of vanadium to that of a template in tunichrome synthesis.

Vanadium in Amanitae

Several species of the *Amanita* family sequester vanadium, with the fly agaric (*Amanita muscaria*) exhibiting the highest concentrations (up to 325 mg kg^{-1} dry weight; Meisch *et al.* 1978). The vanadium has been isolated in the form of a low-molecular weight compound, *amavadine*, with a 'naked' (i.e. without the doubly bonded vanadyl oxygen) V(IV) center linked to carboxylate and η^2-bonded hydroxylamide groups of *N*-hydroxyimino-α,α'-dipropionic acid (Bayer 1987). Amavadine itself has not yet been crystallized, but its structure is presumably described by the synthetic model **22** in Figure 6 (Carrondo *et al.* 1988). The function of this eight-coordinate, very stable (the overall complex formation constant amounts to 10^{25}) compound is not known. Since amavadine is easily and reversibly oxidized to its V(V) form, a role as a mediator for electron transport is suggestive: the redox potential for the couple amavadine



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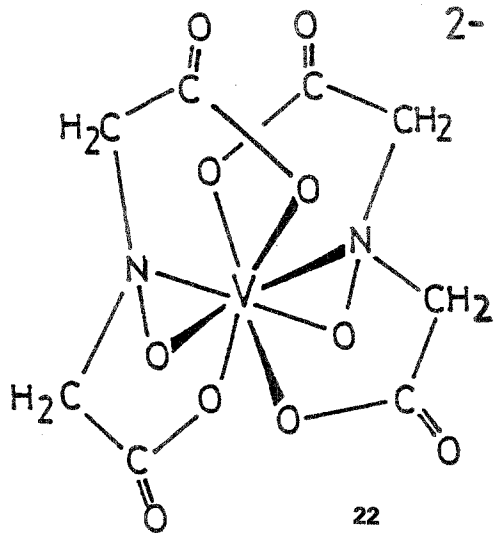

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Figure 6. Hydoxy-DOPA based oligopeptides (tunichromes, **21**) in ascidians may serve as reducing and sequestering agents for vanadium in these animals. R and R' are H and/or OH. The structurally characterized model compound (**22**), a hydroxyiminodiacetate complex of V(IV), is closely related to amavadine, a V(IV) complex isolated from toadstools belonging to the genus *Amanita*. Amavadin itself contains hydroxyiminodipropionate.

(V(V)) + e → amavadine (V(IV)) in water is +0.53 V versus SCE (Nawi & Riechel 1989) and hence is within the range of biological redox agents.

Concluding remarks

The vanadate/phosphate antagonism renders vanadium a regulatory element, possibly essential for all organisms. Chiefly in its oxidation states $+V(H_2VO_4^-)$ and $+IV(VO^{2+})$, this metal may therefore be considered a generally important trace element. *Specific* biological functions of vanadium are scarce and restricted to a small number of groups of organisms. Vanadium might have been used more commonly in earlier stages of evolution and replaced by more effective metals later on. It has been suggested that amavadine is a component of a primitive oxidase formed in remote geological ages and replaced during the evolutionary process by copper oxidases (Fraústo da Silva 1989). It is also known that the phylogenetically younger ascidians accumulate iron instead of vanadium. The two metals are comparable in their chemistry as far as reduction by and coordination to tunichromes is concerned. One of the last elements for which the biological importance has been recognized, vanadium also remains one of the least distributed (with respect to specific functions) in organisms. Nonetheless, new developements and surprising new discoveries may further stimulate investigations into the biochemistry of vanadium. Two examples of promising new developments in this field are the *de novo* synthesis of vanadate-dependent peroxidase in protoplasts of the seaweed Laminaria (Jordan *et al.* 1991) and the extraction of a vanadium protein with bromoperoxidase activity from ascidian vanadocytes (Hawkins 1991), with characteristics very much reminiscent of the haloperoxidases from marine brown algae.

Acknowledgments

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