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COORDINATION CHEMISTRY OF VANADIUM IN BIOLOGICAL SYSTEMS

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CONTENTS

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A. INTRODUCTION

In sharp contrast to most of the first row transition metal ions, the biochemistry of vanadium has been relatively uninvestigated until recently. Sporadic reports dealing with the accumulation of vanadium by certain marine organisms appeared as far back as the turn of the century and the element was found to be essential to many organisms by feeding studies in the 1970s and 1980s [1,2]. However, it was not until the 1977 discovery by Cantley and coworkers that the vanadate ion was a potent inhibitor of the Na^+,K^+ ATPase and their suggestion that endogenous vanadate might act as a regulator of the sodium pump in vitro [3] that a possible biochemical role for vanadium emerged. Subsequent developments have questioned this possibility [4], but it is arguably this first suggestion of a physiological role for vanadium that has caused a resurgence of interest in recent years.

The purpose of this review is to look at the development of vanadium biochemistry since 1983. Prior to 1983 several excellent reviews by Kustin et al. and Chasteen are available and the reader is referred to them for much of the previous work [6,7]. Our intent is to examine the interaction of vanadium with biological systems from the perspective of the coordination chemist, that is with particular emphasis on structure and the reactivity of the metal in various biological systems. Because of the tremendous volume of work now available concerning the biochemistry and physiology of vanadium, it will be impossible to do justice to all areas. In particular the environmental [8], toxicological [8,9] and many physiological effects such as the vanadium-catalyzed oxidation of NAD(P)H (see, for example, refs. 10–13) will not be covered. In addition, this review will not address those aspects where vanadium exerts its action by virtue of the fact that the tetrahedral vanadate ion is a mimic for phosphate [3–5]. However, it now appears that in many phosphate-recognizing enzymic systems, the mode of inhibition by vanadate may have a far more complicated basis than its superficial resemblance to phosphate since the most potent inhibitor of many phosphate-metabolizing enzymes is the cyclic vanadate tetramer as opposed to the simple vanadate monomer [14,15].

Part I. Recent advances in the coordination chemistry of vanadium

In order to understand how vanadium might function in relatively complex biomolecules it is incumbent on us to understand its basic coordination chemistry with simpler ligands. While vanadium can exist in at least six oxidation states, only the three highest, i.e. +3, +4, and +5, are important in biological systems. Oxidation states below +3 are generally too reducing

to exist in aqueous environments at neutral pH and will not be considered here. Of the three remaining, the +4 and +5 states, the chemistry of which is often associated with the oxycations VO^{2+} and VO_2^+, are by far the most common. Part I is a brief and selective look at some recent vanadium coordination chemistry that is important to our understanding of the biochemistry of the vanadium. An outline of the aqueous, uncomplexed chemistry of vanadium in these oxidation states can be found in reviews by Chasteen [7], and Boas and Pessoa [16] and a discussion of the oxidation–reduction reactivity of vanadium complexes in aqueous solution is found in a review by Butler [17].

B. VANADIUM–SULFUR COORDINATION

Until recently, vanadium–sulfur complexes had not been well-characterized. However, because of the widespread occurrence of the thiolates in metalloproteins and the evidence for V–S coordination in the newly discovered vanadium-nitrogenase (discussed below), vanadium–sulfur complexes are now of considerable interest. Mononuclear homoleptic thiolates of V(III) have been prepared by Randall and Armstrong using the sterically demanding 2,4,6-tri-isopropylbenzene-thiolate (tipt) ligand [18]. Although V(III) complexes are typically octahedral, these relatively crowded species adopt a trigonal bipyramidal geometry. The structure of pentacoordinate $[V(tipt)_3(thf)_2]$ reveals a V–S bond length of 2.32 Å (Fig. 1) [18], a value

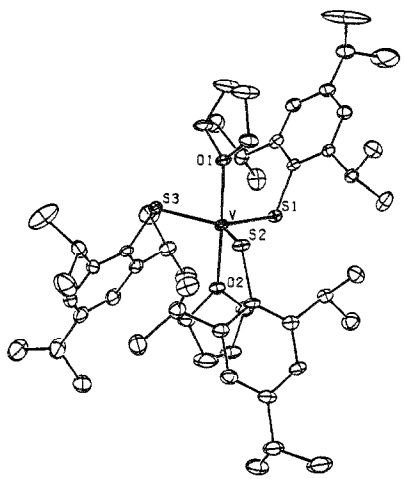


Fig. 1. Structure of $[V(tipt)_3(thf)_2]$ showing the 30% probability thermal ellipsoids and atom-labelling scheme. Hydrogen atoms are omitted for clarity. Selected interatomic distances (Å) and angles (°) are as follows: V–S(1) 2.308(1), V–S(2) 2.334(1), V–S(3) 2.320(1), V–O(1) 2.123(2), V–O(2) 2.109(2), S(1)–V–S(2) 116.11(3), S(1)–V–S(3) 116.33(3), S(2)–V–S(3) 127.30(3), S(1)–V–O(1) 93.76(6), S(2)–V–O(2) 92.71(6), S(3)–V–O(2) 93.07(6), O(2)–V–O(1) 176.78(8). Reproduced with permission from ref. 18.

similar to that found for the Mo(II)–S bond [19]. Novel di- and higher nuclearity metal–metal bonded, V(III) clusters result when chelating, non-sterically demanding thiolates such as ethanedithiol are used. However, these species may have little biological significance [20,21].

Vanadium(IV) thiolates have also been isolated and characterized in recent years by the Christou group and others [22–25]. Typical five-coordinate square pyramidal vanadyl complexes can be produced using either ethanedithiol or l-cysteine methylester. The V–S bonds average 2.35 Å and the V=O bond 1.62 Å. The small but significant lengthening of the V=O bond (expected >1.60 Å for 5-coordinate species) has been attributed to π-interactions between the sulfur lone pairs and the metal *d*-orbitals, resulting in increased delocalization of the vanadium *d* electron. As expected, these vanadium-thiolates are harder to oxidize and easier to reduce than their nitrogen- or oxygen-coordinating analogs.

Tetrathiovanadate(V), $[VS_4]^{3-}$ [26,27], is a convenient precursor to thiobridged vanadium–iron clusters such as the cubane type $[VFe_3S_4Cl_3(DMF)_3]^-$ cluster and the nearly linear $[VFe_2S_4Cl_4]^{3-}$ ($\angle$ Fe–V–Fe = 172.9°) cluster [27,28] (Fig. 2).

Kovacs and Holm have recently shown that (Me_4N)-$[VFe_3S_4Cl_3(DMF)_3]\cdot 2DMF$ can be prepared by reaction of the linear trinuclear $[VFe_2S_4Cl_4]^{3-}$ cluster with $FeCl_2$ in DMF [28–30] (Fig. 2). The $(VFe_3S_4)^{2+}$ core is isoelectronic and nearly isostructural with the $(MoFe_3S_4)^{3+}$ core. The ^{57}Fe Mossbauer spectrum and the bond lengths (Fe–Cl, V–O) suggest $[VFe_3S_4]^{2+}$ is best characterized by a delocalized distribution of oxidation states, e.g. V(II½)/3Fe(II½), as opposed to a localized charge distribution of V(IV)/3Fe(II) [28]. The magnetic susceptibility of $(Me_4N)[VFe_3S_4Cl_3(DMF)_3]\cdot 2DMF$ obeys near Curie behavior ($\mu = 3.87$), indicative of an $S = 3/2$ ground state [28]. Some of these features are remarkably similar to the vanadium–iron–sulfur cluster extracted from

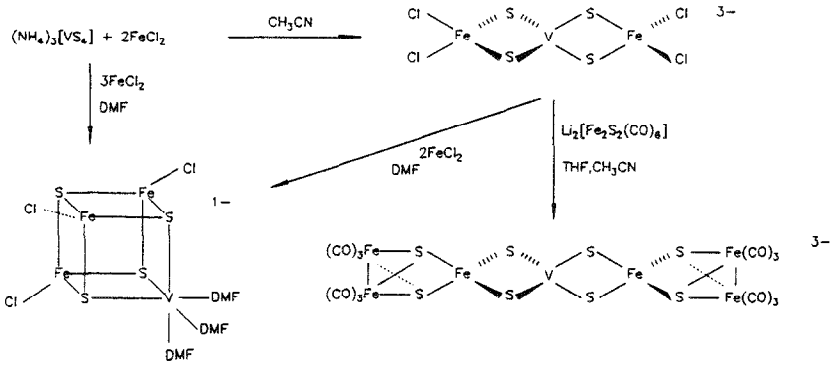


Fig. 2.

vanadium-nitrogenase (see below), which strongly points to the presence of a vanadium–cubane-type cluster in the enzyme. The lability of the terminal chloride and DMF ligands of $(Me_4N)[VFe_3S_4Cl_3(DMF)_3]\cdot 2DMF$, which have been replaced by thiolate, phosphine and phenoxide ligands, suggest that other novel cluster environments can be synthesized, possibly with exceptional reactivity. In fact, Ciurli and Holm have recently reported the insertion of the $[VFe_3S_4]^{2+}$ and the $[MoFe_3S_5]^{3+}$ clusters into the semi-rigid trithiolate-containing cavitand ligand, 1,3,5-tris((4,6-dimethyl-3-mercaptophenyl)thio)-2,4,6-tris(*p*-tolylthio)benzene (LS_3) [31] (Fig. 3). This ligand coordinates exclusively to the iron sites, leaving the vanadium and molybdenum sites available for reactivity. The striking difference between the vanadium-coordinated tricyano complex, $[VFe_3S_4(LS_3)(CN)_3]^{4-}$ and $[VFe_3S_4(LS_3)(DMSO)_3]^{-}$ is the 0.5 V shift in reduction potential [31].

In addition to the VFe_3S_4 cubane cluster, other novel clusters are also known, including the linear $[VFe_6S_8(CO)_{12}]^{3-}$ cluster (Fig. 2), and the double cubane-type $[V_2Fe_6S_8Cl_4(edt)_2]^{4-}$ (edt, ethane-1,2-dithiolate) [29]. Ti_3VS_4 and the mineral sulvanite, Cu_3VS_4, are also examples of thiobridged vanadium-metal clusters present in extended lattice networks, although neither has been identified as a discrete monomeric cluster. The mineral patronite $(VS_4)_n$ is a chain of pairs of vanadium(IV) ions bridged by side-on bound disulfide ligands [26].

C. VANADIUM–NITROGEN COORDINATION

Although the number of vanadium–nitrogen complexes isolated and characterized over the past several years is certainly very large, only a few are of

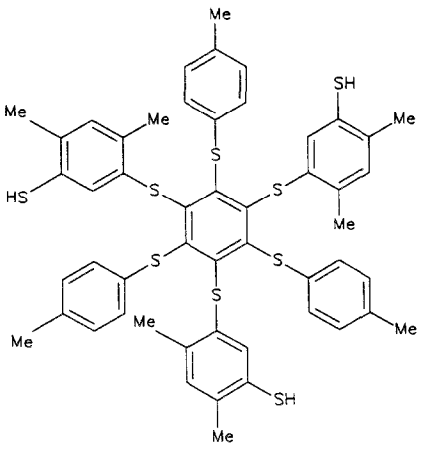


Fig. 3.

more than passing biological relevance. Very few homoleptic V–N species are presently known. One well-characterized example is the V(IV) sepulchrate reported by Sargeson and coworkers [32] (Fig. 4). This is one of the most prominent examples of a 'bare' (i.e. lacking oxide ligands) V(IV) complex due to its stability in aqueous solution and nearly trigonal prismatic structure. Another homoleptic V–N species, which may be of use as an EXAFS model for V–histidine interactions, is the vanadium(III) bis(trispyrazolylborate) [33]. This V(III) complex, $L_2V^+X^-$ where L = 3,5-dimethylpyrazolylborate, is air stable and its X-ray structure has been determined (i.e. V–N bond length of 2.08 Å). In addition to the 'full sandwich' complex, a variety of 'half sandwich' complexes of V(III) and V(IV) with this ligand have been synthesized and characterized [34]. Complexes using the topologically similar ligand, 1,4,7-trimethyl-1,4,7-triazacyclononane have been reported by Wieghardt and coworkers [35]. The structures of many of these species have been determined by X-ray crystallography. Of particular interest is the vanadium(IV) complex LVOCl(DMF), where L is 3,5-dimethylpyrazolylborate (Fig. 5). This octahedral species can be oxidized electrochemically or with peroxides to give a V(V) complex [34]. The V(V) complex with peroxide bound may be a valuable structural model for the active site in the bromoperoxidases since it contains a peroxide and a halide coordinated to a vanadium(V) center. A vanadium(IV) complex with imidazole ligands has been reported as an EXAFS model for the reduced, inactive form of bromoperoxidase [36].

An unrelated complex of current interest, in light of the vanadium-containing nitrogenase, is the hydrazido complex $[VCl_2(H_2NNMePh)_2(NNMePh)]Cl$, which shows simultaneous coordination of side-on bound hydrazine and end-on bound hydrazide [37]. These species are important potential intermediates in dinitrogen reduction. However, one wonders

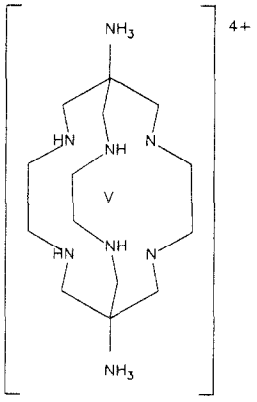


Fig. 4.

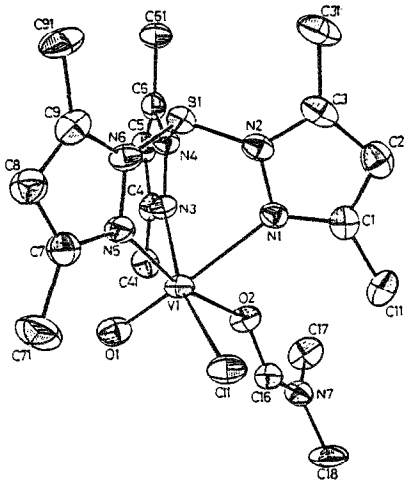


Fig. 5. The vanadium(IV) complex LVOCl(DMF) where L is 3,5-dimethylpyrazolylborate. Reproduced with permission from ref. 34.

whether vanadium or molybdenum serve a unique function in nitrogenase since an iron-only nitrogenase is now known [38].

Wieghardt and coworkers have also used a variety of nitrogen-containing chelates to show that hydrolysis of V(III) can lead to formation of a stable $V_2(\mu\text{-}O)(\mu\text{-}MeCO_2)_2^{2+}$ core [39]. Interestingly, this complex displays a weak ferromagnetic exchange interaction rather than the strong antiferromagnetism seen with the iron and manganese analogs [39]. The magnetic properties of these dimers are discussed in detail by Wieghardt and coworkers [39]. Although, as yet, this core structure has not been found in any vanadium-containing biological molecule, it is a very common and stable entity found in several iron and manganese metalloproteins such as ribonucleotide reductase, hemerthyrin, and pseudocatalase [40–42]. Characterization of the vanadium core in model complexes may allow it to be identified in a protein system should it exist.

D. VANADIUM–OXYGEN COORDINATION

A number of potentially ligating, oxygen-containing functional groups are found in proteins. The carboxylate of glutamic and aspartic acids, the alkoxy group of deprotonated alcohols, such as serine and threonine, and the phenolate oxygen of tyrosine are members of this group. Synthesis of models containing these types of coordination have been actively pursued over the last few years and have provided a variety of insights into vanadium chemistry that may have particular relevance to biological systems.

N-Hydroxylated ligands, although potentially ambidentate, are often bound to metals via oxygen coordination. The reaction of vanadium with these ligands has important implications with respect to the coordination modes of the natural product Amavadin [43] and the possible binding and transport of vanadium by hydroxamate siderophore-like molecules from marine bacteria [44–48]. Reaction of vanadium(V) complexes with the simplest member of this group of ligands, hydroxylamine, is quite complex [49] (Fig. 6). In alkaline solution the final species is a V(I) nitrosyl, as characterized by the nearly linear V–N–O coordination and the N–O stretching frequency of 1530 cm^{-1}. The η^2, side-on coordination of the N–O unit in species (3) (Fig. 6) is unusual but has been characterized in related molybdenum complexes [50,51] and is proposed to occur in Amavadin as well [52,53]. Side-on bound hydroxylamine is formally analogous to side-on bound hydrogen peroxide, both of which produce seven-coordinate vanadium(V) complexes (see below). In mildly acid solution an exclusively N-bonded hydroxylamine compound (i.e. species (2) in Fig. 6) is isolated [49].

Vanadium(V) also binds to a related series of ligands, the hydroxamates. Hydroxamates have long been known to be powerful bidentate chelating

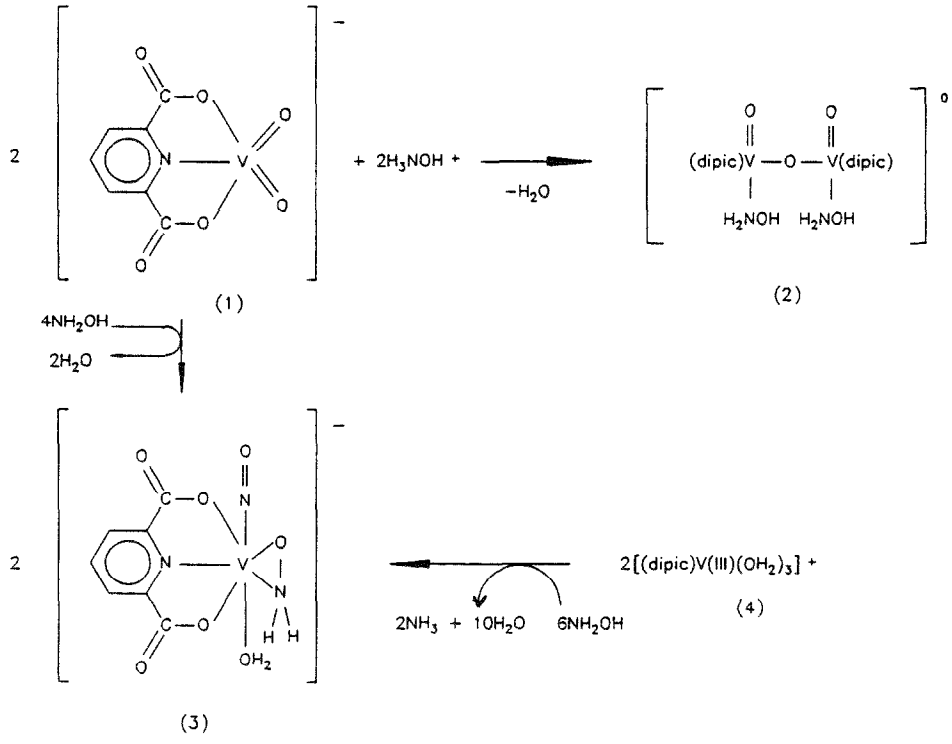


Fig. 6.

agents for a variety of metals, particularly Fe(III). They are common constituents of siderophores [54]. Disubstituted hydroxamic acids have been used as colorometric reagents for the determination of trace quantities of V(V) due to their intense blue color (λ_{max} 530 nm, ε 4000 M^{-1} cm^{-1}) [55,56]. The species responsible for the color was suggested to be the bishydroxamate complex of the VO^{3+} moiety [56]. A recent X-ray structure has appeared confirming this formulation [57]. A structure has also appeared on the VO^{3+} complex of a deprotonated monosubstituted hydroxamate. This complex appears as a trinuclear cluster with an N–O bridged vanadium(V) center [58]. Vanadate also coordinates desferrioxamine, the tri-hydroxamate siderophore from *Streptomyces pilosis* [59–61].

The carboxylate moiety can coordinate to metals in a variety of ways. Simple monodentate binding, usually in the form of an aminocarboxylate chelate, is widespread. Acetato and benzoato bridged complexes of V(III and IV) are also common, while mononuclear carboxylato complexes of V(V) have only recently been reported [62,63]. These complexes, which contain bidentate carboxylate coordination (see Fig. 7), have been suggested to be models for the vanadium in the binding site of bromoperoxidases. There is evidence that the chelate rings in the pentagonal bipyramid structure found in the solid state probably do not persist in solution.

Sakurai and Tsuchiya have recently proposed that a series of dicarboxylato ligands (i.e. oxalate, glutarate, succinate, malonate) complexed to vanadyl

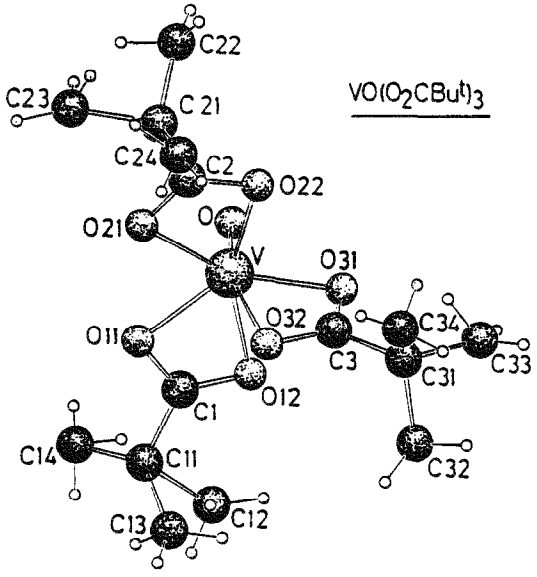


Fig. 7.

react by the scheme shown in Fig. 8 and as such are mimics of vanadium bromoperoxidase [64]. The proposed mechanism involves reduction of hydrogen peroxide by V(IV), forming hydroxyl radical (reaction (1)), subsequent reactions of V(V) with hydrogen peroxide and bromide forming $[V(V)(OH)(O_2)Br]^-$ (reactions (2) and (3)) and finally reaction of two equivalents of hydroxyl radical with $[V(V)(OH)(O_2)Br]^-$ to produce HOBr (reaction (4)). Several steps in this proposed mechanism are energetically unfavorable; for example, excess bromide in solution will undoubtedly react with hydroxyl radical instead of having to invoke the proposed bromoperoxo–V(V) complex. Another problem is that vanadyl bromoperoxidase apparently is not oxidized by hydrogen peroxide and does not catalyze the bromination of MCD (see below). Thus it is highly unlikely that these vanadyl complexes are true vanadium bromoperoxidase mimics, especially proceeding through the formation of hydroxyl radical.

Vanadium peroxo complexes continue to be studied and are going to be important models for the haloperoxidase enzymes. Vanadium(V) forms many stable peroxo complexes both with and without auxiliary heteroatom ligands. Despite the potential for a variety of coordination modes, all the simple peroxo complexes structurally characterized thus far display seven-coordinate, pentagonal bipyramidal vanadium(V) with symmetrically coordinating η^2-peroxides, in the pentagonal plane (see, for example, refs. 65 and 66) (Fig. 9). With alkyl peroxides, however, the peroxide triangle is not symmetric i.e. the V–O bonds are 1.872 and 1.999 Å in [dipic]VO[OOtBu]·H_2O [67]. All the vanadium peroxide complexes are characterized by a ν_{O-O} at 850 cm^{-1} and modestly intense ($\varepsilon = 2\text{–}3 \times 10^2$ $M^{-1}\,cm^{-1}$) peroxo LMCT band in the range of 300–460 nm that give rise to the red to yellow colors of these complexes [68]. The lack of a discernible band in this region for

$$V(IV)O^{2+} + H_2O_2 \rightarrow V(V)O_2^+ + \cdot OH + H^+ \quad (1)$$

$$V(V)O_2^+ + 3\,H_2O_2 \rightarrow [V(V)(OH)_2(O_2)_2(OH_2)_2]^- \quad (2)$$

$$[V(V)(OH)_2(O_2)_2(OH_2)_2]^- + Br^- \rightarrow [V(V)(OH)(O_2)_2Br]^- + OH^- + 2\,H_2O \quad (3)$$

$$[V(V)(OH)(O_2)_2Br]^- + 2 \cdot OH \rightarrow HOBr + [V(V)O(OH)(O_2)_2]^{2-} + H^+ \quad (4)$$

$$HOBr + \text{substrate} \rightarrow \text{S-Br} + H_2O \quad (5)$$

In the absence of substrate

$$HOBr + H_2O_2 \rightarrow Br^- + O_2 + H^+ + H_2O \quad (6)$$

Fig. 8.

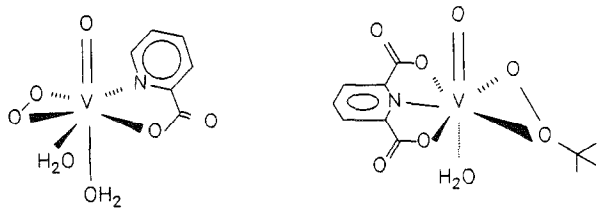


Fig. 9.

the V(V)-dependent bromoperoxidase enzyme may be significant although structural/electronic effects on the position of the peroxo LMCT have yet to be completely investigated.

Considerable work has appeared concerning the role of the coordinated peroxo group in a variety of oxidation and oxygenation reactions [69,70]. Based on an analysis of the products, it is anticipated that these reactions can involve either homolytic cleavage of the peroxo bond to yield the diradical V(IV)–O·, or heterolytic cleavage initiated by nucleophilic attack on the electrophilic oxygen. The specific pathway may depend in part on the degree of asymmetry in the vanadium–peroxidic triangle.

Vanadium–phenolate interactions have been implicated in a variety of vanadium biomolecules. Homoleptic V(III)-phenolates can be prepared using sterically demanding aryloxides such as 2,6-diisopropylphenolate or 2,6 dimethylphenolate. Under varying conditions, six-coordinate pseudooctahedral, four-coordinate tetrahedral (both mononuclear and bridged dinuclear), and planar three-coordinate species can all be produced [72–74]. This demonstrates a rather unexpected degree of coordination flexibility for this ion. In general, the vanadium in these complexes is readily oxidized to the +4 and +5 states but is reduced only with great difficulty [72]. This is the expected pattern since the hard phenolate oxygen donor can be expected to stabilize the higher oxidation states of the metal preferentially.

Cooper et al. have isolated and, in many cases, crystallographically characterized a complete set of V(III), V(IV) and V(V) complexes of catechol [75]. The green V(III) triscatechol complex exhibits the expected octahedral geometry. Two V(IV) catechol species are formed. The expected square pyramidal biscatechol vanadyl complex is pale blue ($\varepsilon \sim 50$), but addition of excess catechol produces an intensely blue V(IV) triscatecholate octahedral complex, lacking a pendant oxo group. Oxidation of the V(IV) complex gives a stable V(V) triscatecholate which is one of only two species that we are aware of which contains a 'bare' V(V) center [75,76]. Bare V(IV) coordination was once considered to be rare, however many complexes have been identified since the first example, i.e. the trigonal prismatic tris(1,2 dicyanoethylene-1,2-dithiolate)vanadium(IV) complex [77]. Oxygen-ligation most commonly characterizes the bare V(IV) moiety as in V^{IV}(acetylacetonate)$_3^+$ complexes

[78,79] and mixed ligand V^{IV}(catecholate)(acetylacetonate)$_2$ complexes [80], although other V^{IV}(dithiolene)$_3^{2-}$ [81,82] and, of course, the nitrogen-bonded V(IV) sepulchrate [32] are well known.

Vanadium(IV and V) phenolates have also been characterized in the form of multidentate heteroligand chelates, of which a particularly well-studied family is shown in Fig. 10. The results of the studies on these and other systems can be summarized as follows [83,84].

(1) The chelate chemistry of vanadium(V) is not limited to the VO_2^+ moiety as has often been supposed. Rather it displays a rich chemistry involving VO^{3+}, $VO(OH)^{2+}$ and bare V^{5+} as well as the ubiquitous VO_2^+.

(2) The oxo group normally associated with V(IV) and V(V) can be removed by protonation and this protonation can proceed under physiologically relevant conditions. Floriani and coworkers first proposed that a dioxovanadium(V) center could be protonated to give a $VO(OH)^{2+}$ moiety which could be viewed as the inorganic analog to a carboxylic acid [85]. Using the 8-hydroxyquinolinato (Q) complex of VO_2^+, they demonstrated the value of that analogy and proposed protonation equilibria such as that shown in Fig. 11 for which they determined pK_a values of 6.3 and 3.4. Bonadies and Carrano [83] suggest that the diprotonated species is better formulated as L_2VO+H_2O. With the EHPG ligand, the VO^{3+} moiety can be produced by successive protonation and ultimate loss of an oxo group as water from a VO_2^+ center via the formation of the $VO(OH)^{2+}$ intermediate. The mea-

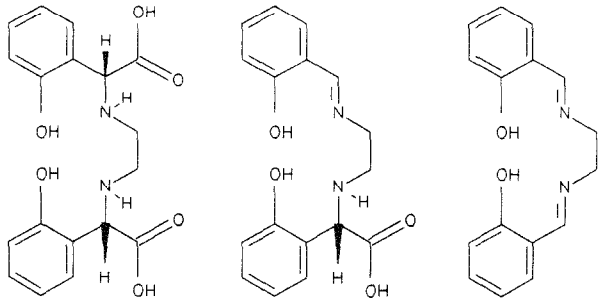


Fig. 10.

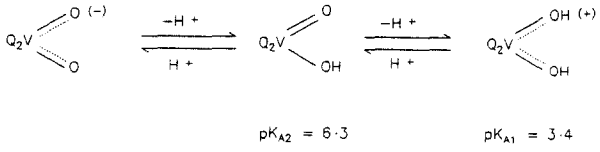


Fig. 11.

sured pK_a values of this process of 6.1 and 3.5, respectively, are remarkably similar to those reported by Floriani and coworkers. The fact that the VO_2^+ unit in a variety of ligand environments clearly can undergo protonation in the physiological region has important biological implications that have remained largely unappreciated.

The protonation of vanadium oxo groups can be extended to some vanadyl species as well [84]. The oxo group of the vanadyl ion can also be removed by dehydration with oxophilic reagents (e.g. $SOCl_2$, PCl_5) or by displacement with strong chelating, oxygen donors to produce non-oxo or 'bare' V(IV) centers [75,86,87]. The only known example of a 'bare' V(V) center also arise from displacement of the oxo groups of vanadate by catecholates and phenolates [75,76].

(3) The stabilities of the three oxidation states of vanadium can be strongly modified by ligation, solvent and pH. For example, electrochemistry has been used to show that square pyramidal, coordinatively unsaturated V(IV) complexes (Fig. 12, **A**) are more difficult to oxidize than their coordinatively saturated octahedral counterparts (Fig. 12, **B**) [88]. This reflects the coordination preferences of the harder V(V) center which strongly favors six coordination. The ECEC square mechanism which accompanies electron transfer between **A** and **B** demonstrates this effect nicely.

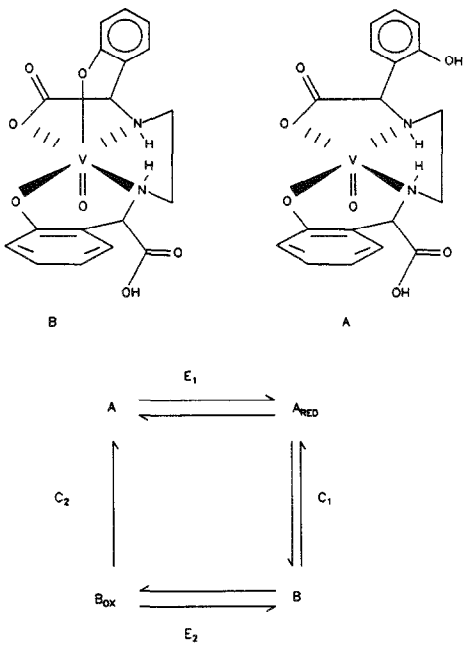


Fig. 12.

(4) While under the appropriate conditions vanadium(III, IV, or V) complexes can each be isolated in a stable form, under other conditions, several oxidation states may coexist. The acid-promoted disproportionation of V(IV)O(SALEN) is a salient example [84]. Under anaerobic conditions in non-coordinating acids, V(IV)O(SALEN) undergoes disproportionation to yield a V(III)(H_2SALEN) species and V(V)O(SALEN). However, in the presence of the coordinating chloride ion, the V(III) species is unstable with respect to oxidation by V(V) and undergoes a back electron transfer to yield V(IV)Cl_2(SALEN) and V(IV)O(SALEN). This behavior formed the basis of a suggestion that a similar reactivity could explain how ascidians produce V(III) starting from V(V) and tunichrome type ligands.

(5) Finally, it is now well established that bare vanadium(IV), bare vanadium(V) and the VO^{3+} ion complexed to phenolate ligands all display LMCT bands in their visible spectra, giving rise to intense blue-violet colors. These colors provide a means of distinguishing bare V(IV and V) and VO^{3+} from the more common VO^{2+} and $VO_2{}^+$ ions, which do not manifest intense LMCT bands. The intensity of the absorption band (i.e. ε 2000–10 000 M^{-1} cm^{-1}) typifies these complexes, not the energy of the absorption band, as many simple vanadyl complexes are pale blue ($\varepsilon \sim 50$). The failure to distinguish between mere color and characteristic spectral features (i.e. band maxima and intensity) has led to much confusion in the literature since it has frequently (and erroneously) been assumed that a blue coloration is characteristic of the vanadyl ion. This confusion has been exacerbated by the failure to quantitate EPR signals presumed to be due to vanadium(IV) or to perform the required magnetic measurements.

E. OTHER CATEGORIES OF VANADIUM COMPLEXATION OF RELEVANCE TO BIOLOGICAL SYSTEMS

Vanadium–alkoxo complexation has also begun to be investigated. Early results indicate that a deprotonated alcohol function strongly favors the +5 oxidation state and is frequently involved in bridging interactions [89].

A dibromo vanadium(III) tetraethyleneglycol complex, [V(III)(teg)$(Br)_2$]Br, has been obtained by reduction of vanadate by HBr in the presence of tetraglyme ($CH_3O(CH_2CH_2O)_4CH_3$) [90]. The reaction is accompanied by loss of the terminal methyl groups of tetraglyme. [V(III)(teg)$(Br)_2$]Br has a pentagonal-bipyramid structure with the tetraethyleneglycol ligand occupying the five equatorial sites, with two axially coordinated bromide ions. [V(teg)$(Br)_2$]$^+$ is re-oxidized by dioxygen, presumably forming a V(V)=O complex. If a continuous stream of HBr and air passes through a solution of vanadate and tetraglyme in dichloroethane, a catalytic system is formed that oxidizes bromide by dioxygen, producing bromine. The electron transfer

role of vanadium has possible relevance to vanadium bromoperoxidase, although this system and V–BrPO differ in that V–BrPO requires the presence of hydrogen peroxide for catalytic activity (see below).

The predominant form of vanadium(V) in aqueous solution at neutral pH, is vanadate ($H_2VO_4^-/HVO_4^{2-}$) or one of its oligomers (e.g. $H_2V_2O_7^{2-}$, $V_4O_{10}^{4-}$, $H_2V_{10}O_{28}^{4-}$, etc.) depending on the pH and vanadium(V) concentration. Vanadate readily reacts with a variety of small molecules, forming a diversity of complexes, many of which are in rapid equilibration with each other. ^{51}V NMR has proved to be an exceptionally useful technique to characterize such vanadium(V) complexes. The large magnetic moment of ^{51}V, its high natural abundance (99.76%) and its rapid relaxation properties contribute to its applicability as a selective probe of vanadium(V) and low-spin vanadium(−1) complexes, despite the fact that it has a nuclear spin of 7/2. ^{51}V NMR is an excellent diagnostic tool for detailed investigations of vanadium(V) coordination environments because the chemical shifts are very sensitive to the nature of the coordinated ligands and the coordination environment (see ref. 91 for a review). The chemical shifts span a range from +2000 ppm (vs. $VOCl_3$) for thiovanadates(V) to −800 ppm for peroxovanadium(V) complexes. Low-valent vanadium(−1) complexes that have d^6, low-spin electronic configurations are also observable by ^{51}V NMR, but this oxidation state probably does not have significant biological applications.

One application of ^{51}V NMR spectroscopy of relevance to biochemical investigations is the study of vanadate interactions with a wide variety of buffer salts. High concentrations of vanadate–buffer complexes are formed in bicine (*N*,*N*-bis(2-hydroxyethyl)glycine), bis-tris (bis(2-hydroxyethyl)imino-tris(hydroxymethyl)methane) and tricine (*N*-tris(hydroxymethyl)methyl-glycine) [92]. Weak complexes are also formed with Tris (tris(hydroxy-methyl)aminomethane), even at a low vanadate concentration [92,93]. Thus, depending on concentration, pH and the magnitude of the complexation constant, vanadate may not be present in solution in the desired or expected form. This is a consequence of the coordination chemistry of vanadate that has not been thoroughly appreciated. For example, investigations of vanadate inhibition of phosphate-metabolizing enzymes are strongly affected by the nature of the buffer salt [92]. The degree of vanadate oligomerization or complexation by phosphate, reactions that are also conveniently studied by ^{51}V NMR, can also have tremendous effects on the course of a reaction under investigation [94]. Clearly, the recent reports have shown that one must be acutely aware of the complexation properties of vanadate in biochemical investigations.

Another area of potential relevance to the biological chemistry of vanadium is the oxygen-atom transfer reactivity of vanadium species. Compared with other transition metal ions, not many examples of true oxygen-atom transfer

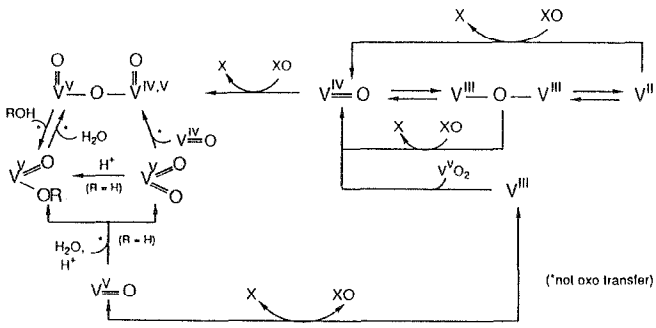


Fig. 13. Double headed curved arrows indicate that the transformations occur in both directions. Non-oxygen atom transfer processes are marked with an asterisk. Reproduced with permission from ref. 96.

reactions are known for vanadium [95]. Holm has summarized the oxo-atom transfer reactivity that interconnects V(II,III) and the oxo species V(IV)O, V(V)O, $V_2(V)O_3$, $V_2(IV,V)O_3$ and $V(V)O_2$ which is shown in Fig. 13. In this generalized scheme, which includes all known oxo transfer reactions of vanadium (excluding vanadium peroxide), the reactions fall into two types, either as primary oxotransfer reactions or as electron transfer reactions with μ-oxo bridge formation [96]. The main reactions are

$V(II) + XO \rightarrow V(IV)O + X$

$V(III) + XO \rightarrow V(V)O + X$

and

$V(V)O + X \rightarrow V(III) + XO$

$V(V)O_2 + V(III) \rightarrow 2\ V(IV)O$

$V(II) + V(IV)O \rightarrow V(III)\text{–}O\text{–}V(III)$

and

$V(II) + V(V)O \rightarrow V(III) + V(IV)O$

where XO/X is the oxo donor/acceptor (e.g. iodosyl benzene, *m*-chloroperoxybenzoic acid, triphenylphosphine oxide). The conversion of V(IV)O or V(V)O to $V(V)O_2$ by strong oxo-donors are not primary oxotransfer reactions, but are examples of the redox reactions

$[V(V)OCl_4]^- + XO(PhIO,MCPBA) \rightarrow [V(V)O_2Cl_2]^- + Cl_2 + X(PhI,MCBA)$

$[V(IV)OCl_4]^{2-} + PhIO \rightarrow [V(V)O_2Cl_2]^- + PhI + Cl^- + \tfrac{1}{2}\ Cl_2$

Part II. Biosystems

F. ORGANISMS THAT ACCUMULATE VANADIUM

(i) Amanita muscaria

One of the simplest and yet most interesting vanadium-containing natural products is Amavadin. It has been known for some time that the common

mushroom *Amanita muscaria* or fly agaric, concentrates vanadium to a considerable extent. However, it was not until 1972 that Bayer and Kniefel isolated the vanadium-containing compound and gave it the name Amavadin [43]. Based on elemental analysis, IR, EPR, and chemical reactivity, they assigned Amavadin a structure in which a vanadyl ion was coordinated by two molecules of *N*-(l-1-carboxyethyl)-*N*-hydroxy-l-alanine (or *N*-hydroxy-2,2′-iminodipropionic acid) (Fig. 14) [97]. Later, these same authors were able to achieve the total synthesis of the complex [98]. The proposed structure was supported by potentiometric titration data on the vanadyl complex with *N*-(l-1-carboxyethyl)-*N*-hydroxy-l-alanine and related ligands, the presence of a purported V=O stretch at 980 cm^{-1} and EPR data. Subsequently, several new results suggested that the proposed structure was incorrect. In 1987, Bayer et al. measured the stability constants for the vanadyl ion with metal-free Amavadin and *N*-hydroxyiminodiacetic acid ligands as well as their non-*N*-hydroxylated counterparts [52]. Their results showed that the *N*-hydroxylated ligands (i.e. compound (1), Fig. 15) form far stronger and more selective 2:1 complexes with VO^{2+} (i.e. $\log \beta_2 = 23$) than simple iminodiacetates (e.g. compound (2), Fig. 15) which form only 1:1 species (i.e. $\log K_1 = 9.5$). For all the other metal ions examined, i.e. Cu^{2+}, Zn^{2+}, Ni^{2+}, Ca^{2+},

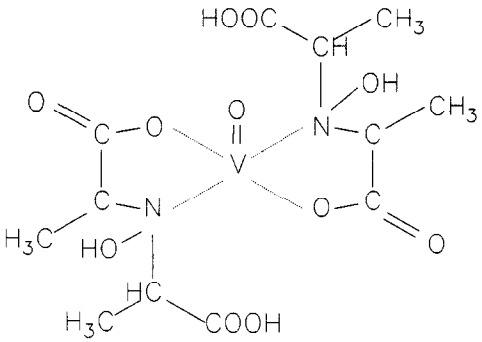
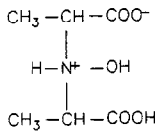
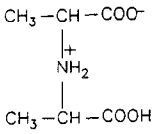


Fig. 14.



(1)

N–hydroxy–2,2'–iminodipropionic acid



(2)

iminodipropionic acid

Fig. 15.

etc., the simple imino ligand forms the more stable complex, compared with the corresponding *N*-hydroxy compound. These observations suggest that the *N*-hydroxy group is important to the overall stability of the vanadium complex. This is also supported by the electrochemical study of Amavadin by Asri Nawi and Reichal who showed that only the *N*-hydroxyl compounds such as the Amavadin ligand lead to reversible electrochemical oxidation of the vanadium [99]. The vanadium complexed to these ligands is also ca. 1 V easier to oxidize than the simple amino analog. Finally, large-angle X-ray scattering (LAXS) experiments suggested that the shortest vanadium–ligand distance in Amavadin is on the order of 1.9 Å. Since the V–O bond in the vanadyl ion is expected to be 1.5–1.6 Å, this work suggests the *absence* of a vanadium oxo group. This suggestion is corroborated by the observation that the IR band at 980 cm^{-1} previously assigned as a V=O stretch in Amavadin itself is not present in the analogous vanadium(IV) complex of *N*-hydroxyiminodiacetic acid even though the two complexes show similar stabilities. Taken together, this data led Bayer et al. to suggest a new structure for Amavadin which features a side-on bound N–O group (Fig. 16).

There is a considerable precedence for this rather unusual structure in the chemistry of hydroxylamine bonding to vanadium (see Part I, above). The proposed structure explains why, even though the hydroxylamino group is deprotonated, only two protons are released upon complexation since the protons are consumed in the formation of water by protonation and loss of the oxo group. The widespread belief that the oxo group is a necessary companion to V(IV) has led to several erroneous assumptions in the literature as is seen, for example, in the chemistry of vanadium catechols. In fact it now appears that protonation and loss of the oxo group as water is a fairly common phenomenon in vanadium chemistry and should be considered

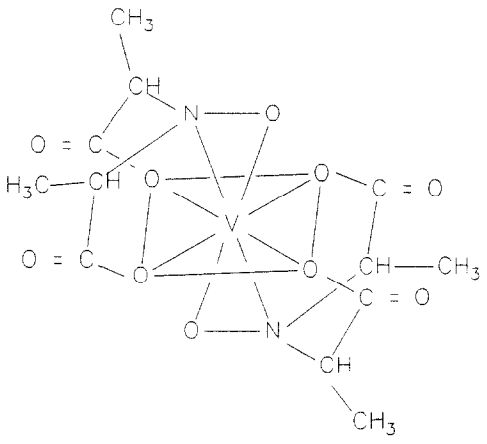


Fig. 16.

whenever strongly coordinating ligands are involved. Proof of the η^2 N–O-coordinated structure requires an X-ray analysis. While such an analysis has not been reported for Amavadin itself, a structure of the analogous V(IV) complex of the related ligand *N*-hydroxyiminodiacetate does indeed show side-on bound N–O coordination [53]. Thus, there is little doubt that the structure of Amavadin itself is analogous to the structure proposed in Fig. 16.

While the puzzle of the structure of Amavadin has been solved, its function remains a mystery. Based on its reversible electrochemistry, Asri Nawi and Reichal have suggested an electron transfer role for Amavadin [99]. Others have pointed to its possible involvement in the circulation of cadmium and vanadium in the environment [100]. Clearly, much more work is necessary on this front in order to establish a clear biochemical function.

(ii) Tunicates

Certainly the most spectacular of the vanadium-accumulating organisms are the tunicates (order *Ascidiacea*). These common, sessile marine animals, also known as sea squirts, have fascinated scientists since the turn of the century when Henze discovered that the blood of such organisms contained large quantities of vanadium. It was originally thought that the vanadium, found primarily in the blood cells known as vanadocytes, was involved in oxygen transport. It is now clear, however, that this is not the case. Our understanding of the nature of the vanadium and its biological role has seen many changes. The history of this evolution up to 1983 has been well presented by Kustin and McCara [101,102]. A recent mini-review which outlines some of the remaining controversies is also available [103].

It is now generally accepted that vanadium, present as a monomeric, pentavalent anion in seawater, is concentrated via a reductive process, as shown schematically in Fig. 17. The vanadium in the blood cells of the

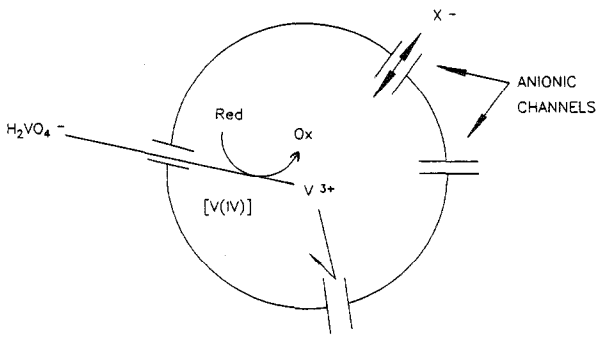


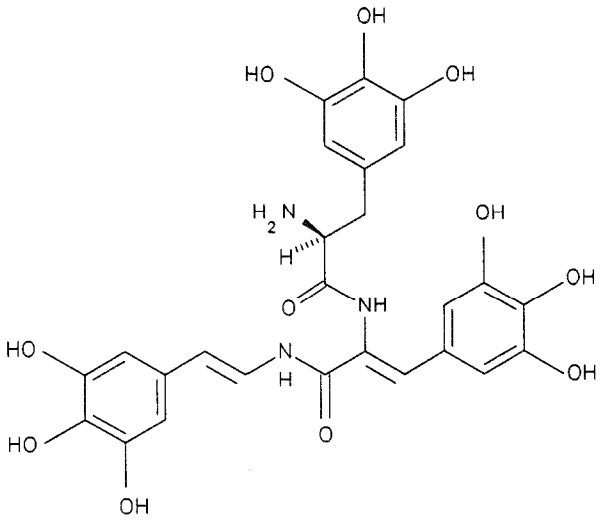
Fig. 17. Tunicate vacuole.

tunicates exists primarily in the trivalent state, although some tetravalent metal is also present. The distribution between the oxidation states appears to be species-dependent. Beyond these two points considerable controversy persists. A lively debate has grown out of an early suggestion that vanadium is contained within cells which maintain an extremely acidic internal pH, originally thought to be equivalent to 1.5 M H_2SO_4 [104]. It was subsequently shown that the methodology on which this estimate was based was erroneous [105]. Later work, using a variety of non-invasive techniques of pH measurement, seemed to indicate a pH near neutrality [106]. However, the validity of some of these techniques has also been questioned. Hodgson and coworkers, based on their results using EPR methods, have given rebirth to the concept that the cells maintain a very low internal pH, near 1.8 [107]. Hawkins and coworkers, however, question both the results and the conclusions [108]. More recently, Hodgson and coworkers reported that they reconfirmed their original work, which indicates a low pH environment for the vanadium [109]. Much of the confusion and controversy undoubtedly rests on the fact that different workers are measuring different physical properties. The pH indicators which suggest a near neutral pH are, in fact, measuring the average whole cell pH. Hodgson's EPR method is really only a measure of the pH sensed by the vanadium(IV). This probably only represents the pH of a small vacuole where the V(IV) is sequestered and might not represent the pH environment sensed by the bulk of the vanadium, which is in the trivalent state and thus EPR-silent at liquid nitrogen temperature. Comparisons cannot readily be drawn between different species of tunicates, since phlebobranch and aplousobranch ascidians may be considerably different in their handling of vanadium. Finally, it is important to avoid comparing the vanadium environment in general ascidian tissue with that found in whole blood. There now seems to be agreement that the two environments differ [108,109].

Another area of confusion, if not controversy, revolves around the role of organic pigments, known as tunichromes, in tunicate vanadium metabolism. In the mid 1970s, it was reported that the yellow green color of the vanadocytes was not due to the vanadium but rather to an organic chromogen named tunichrome. The observation that tunichrome was present in approximately the same molar concentration as vanadium (which may be fortuitous) and was a potent reductant (capable of reducing V(V) to V(IV) in vitro) led to the consideration of tunichrome as an integral part of the vanadium uptake and storage system. Early attempts to isolate and characterize the tunichromes were frustrated by their apparent extreme sensitivity to oxidation and their facile hydrolysis [110]. However, within the last several years, Nakanishi and coworkers at Columbia have finally succeeded in identifying and synthesizing these elusive molecules which are modified tripeptides de-

rived from three hydroxydopa residues (Fig. 18) [111–113]. The presence of several pyrogallol moieties provides both reducing power and the potential for strong metal coordination. Despite the characterization of the tunichromes, their role in the vanadium metabolism of the tunicates remains unclear for the following reasons. In vitro tunichromes only appear able to reduce V(V) to V(IV) and not further to V(III). There are several ways around this apparent problem. First, another biological reductant may reduce the V(IV) to the required trivalent state. This seems somewhat unlikely considering the redox potential which could be expected to be required. A second, more likely possibility is that the V(IV) formed in the initial reduction could become ligated in such a way as to lower its reduction potential. Nakanishi and coworkers have shown that pyrogallol can reduce the V(IV)–bisacetylacetonate complex to a V(III) species [114], and a direct reduction of V(V) to V(III) in the presence of acid and a complexing agent, tetraethylene glycol, has been reported [90]. Pecoraro and coworkers have suggested an alternate mechanism based on work with the phenolate-containing ligand SALEN, where the V(IV) produced by tunichrome reduction could undergo an acid-promoted disproportionation to produce V(III) and V(V) [84]. The V(V) thus produced would then undergo another cycle of reduction by tunichromes (Fig. 19).

A second unresolved issue is the actual location of the tunichrome vis a vis the vanadium. Tunicate blood consists of a number of distinct cell types including the signet ring cells (SR), the morula cells (MC), and compartmental



TUNICHROME

Fig. 18.

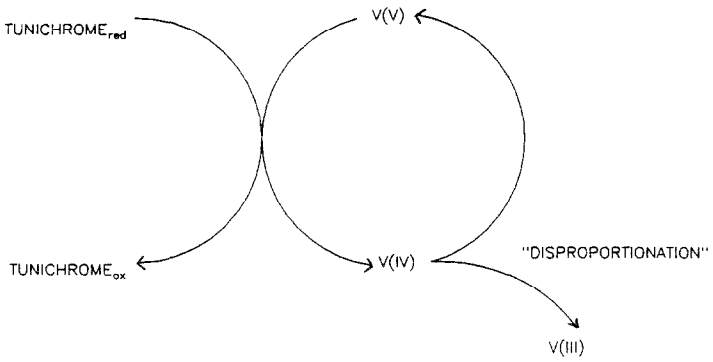


Fig. 19.

cells (CC) as well as several other less common cells. Several studies of various species now indicate that the majority of the vanadium is located in the signet ring cells [113,115]. However, Nakanishi and coworkers have demonstrated that almost all of the *free* tunichrome is present in the morula cells [113]. Of course this does not a priori preclude the possibility that tunichromes are tightly *coordinated* to vanadium in SR cells, but it is most consistent with the idea that the vanadium and tunichrome are stored in separate compartments.

The question of the vanadium coordination environment in the blood cells of tunicates is closely tied to the pH controversy and to the precise location of the tunichromes. Two contrasting conclusions are possible. If the pH of the vanadophores is not markedly acidic, then the V(III) *must be ligated* or it would form insoluble hydroxypolymers. Alternatively, if the V(III) is not ligated, then it *must be in an acidic milieu.* EXAFS, paramagnetic NMR, and EPR have been used to indicate that V(III) is ligated only by water, and sulfate or sulfonate [116]. Carrano and coworkers' work with model complexes shows that, while the tunichromes would be good ligands for V(III) even in acidic solution, based on their stereochemistry they might be expected to yield oxo-bridged dimers [117(a)(b)]. Since such dimers are not consistent with the EXAFS data, this supports the notion that the tunichrome and vanadium(III) are physically separated in intact cells. Further work is still needed to resolve this question. Finally, it should be noted that there is a report of the isolation of another low molecular vanadium binding substance, not a tunichrome, that contains a reducing sugar [118].

G. VANADIUM–PROTEIN INTERACTIONS

(i) Vanadium transferrin

The term 'transferrin' is applied to a class of iron-binding glycoproteins which includes the ovotransferrins, found in large quantities in egg white, the

lactoferrins, originally isolated from human milk, and the serum transferrins, found in the blood plasma of many vertebrates (for reviews, see refs. 119–121). All transferrins are structurally and chemically quite similar, coordinating two moles of ferric ion tightly and reversibly, as well as many other transition metal ions, including vanadium(III,IV, and V). All transferrins have molecular weights of ca. 80 000 Da and consist of single polypeptide chains of approximately 650 amino acids folded into two compact regions, each of which has a high degree of sequence homology and each of which binds a single equivalent of iron. A variety of spectroscopic techniques and other methods indicate that the rough geometry of ferric coordination is octahedral but that the two sites (designated the N-terminal and C-terminal sites) differ subtly in structure [122,123]. X-ray structures were recently completed on rabbit serum transferrin [124] and lactoferrin [125], showing that the amino acid side chains that ligate the iron in each site include histidine, aspartate and two tyrosine residues.

One of the unique features of the transferrins is that a suitable anion must be present in order for iron to bind. In physiological media, the obligatory anion is carbonate or bicarbonate. In the absence of carbonate, numerous other anions, such as oxalate, pyruvate, thioglycolate, etc., promote iron(III) binding, although most simple inorganic anions are ineffective. The function of the synergistic anions may be to 'lock in' the bound metal ion, thereby protecting it from competing reactions such as hydrolysis. In addition to a carboxylic acid moiety, all of the known synergistic anions for transferrin possess a Lewis base functionality within 6.3 Å of the carboxylate group. An 'interlocking sites' model [126] has been proposed and is generally accepted for the interaction of the anion with the metal and protein. Presumably the carboxylate moiety binds electrostatically to the protein and the Lewis base ligates the metal. The X-ray structure, while inconclusive, is consistent with this view.

In addition to ferric ion, the transferrins can bind many other metal ions, some of which have been suggested to have physiological functions. Vanadium-transferrin has been implicated in the metabolism of vanadium both at the very low dietary level and at the high toxic level. Environmental exposure to vanadium (IV or V) reportedly results in the formation of a vanadyl transferrin complex [127]. The nature of this vanadyl transferrin complex is reasonably well understood in light of the extensive study it has received as an EPR probe for the metal binding site in this protein [128,129].

Preparation of the V(III) transferrin complex was reported by Bertini et al. [130]. Little is known about this derivative. It is unique however in that this complex, which might reasonably be expected to be air-sensitive, as are most vanadium(III) phenolates, is in fact reportedly stable to oxidation by either air or hydrogen peroxide. This remarkable result is presumably a dramatic

indication of how ligation can change the properties of metal ions. Interestingly, the higher valent vanadium(IV) vanadyl transferrin *is* oxidized by dioxygen to a vanadium(V) species.

Vanadium(V) transferrins can be prepared either by direct reaction with vanadate or by air oxidation of the vanadyl complex [131,132]. ^{51}V NMR [133,134] and UV difference spectroscopy are highly sensitive tools to probe the nature of the metal binding sites of human transferrin (Tf). At a field strength of 11.7 T, the two vanadium(V)-binding sites in V_2-Tf have ^{51}V chemical shifts at -529.5 and -531.5 ppm versus $VOCl_3$ (Fig. 20). These shifts are assigned to the C- and N-terminal sites, respectively, based on the ^{51}V NMR spectra of vanadate addition to Fe_N-Tf and Fe_C-Tf. Hydrogen peroxide binds to vanadium(V) transferrin as identified by ^{51}V NMR, characterized by a broad resonance at a chemical shift of -600 ppm and UV difference spectroscopy [135]. Tight binding of V(V) to the metal sites of apo-Tf is established by a linear increase of signal area with V(V) concentra-

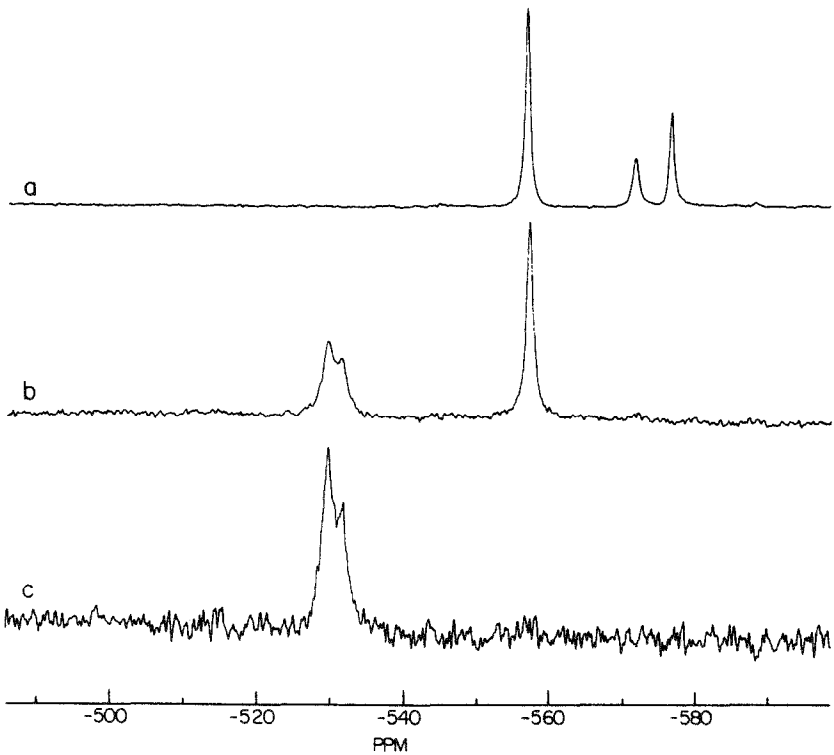


Fig. 20. ^{51}V NMR spectra of vanadate-bound Tf and aqueous vanadate at pH 7.4, 0.1 M Hepes buffer, 131.48 MHz. (a) 0.826 mM aqueous vandate, 26 342 scans; (b) 2.3:1 M/M mixture of V(V) and apo-Tf at 0.826 mM apo-Tf, 205 159 scans; (c) equimolar mixture of V(V) and apo-Tf at 0.34 mM, 200 010 scans. Reproduced with permission from ref. 134.

tion up to an approximate 2:1 stoichiometry, quantitative displacement of protein-bound V(V) by ferric ion, and the lack of the $-529.5/-531.5$ ppm resonances upon addition of vanadate to Fe_2Tf and Ga_2Tf. The chemical shift of the V_C-Tf resonance is independent of pH (5.8–9.0) and temperature (275–310 K), whereas the V_N-Tf resonance varies slightly (1–2 ppm) with pH and temperature. At relatively high V(V) concentrations and at high ratios of V(V)/Tf, conditions under which a large fraction of the V(V) is present as V(V) oligomers, the ^{51}V resonance of Tf-bound V(V) is not observed, possibly due to interference (e.g. exchange) by the oligomeric species. The ^{51}V NMR spectrum of the 20/1 V/Tf shows that the tetrameric vanadate species is substantially broadened relative to the tetrameric vanadate species in a vanadate solution without transferrin, whereas the line width of the monomeric vanadate resonances are the same. This result indicates that the vanadate tetramer, but not the monomer, may be in exchange with protein-bound vanadium [134].

One might expect that the ^{51}V NMR spectrum of vanadium(V) coordinated to a low-symmetry environment or a large macromolecule would display very broad resonances (i.e. several thousand Hz) due to the quadrupolar relaxation. However, in the case of tight V(V) binding to transferrin, the sharpness of the protein-bound resonances (i.e. 400 Hz vs. 100 Hz for $H_2VO_4^-$) is a consequence of the motional characteristics of transferrin, which place the Tf-bound vanadium(V) outside the extreme narrowing limit but within the motional narrowing limit [134]. The results of several different experiments are consistent with the theory for an $I=7/2$ nucleus. The observed signal intensity of transferrin-bound vanadium(V) is only 19% of an equimolar aqueous vanadate sample, since only the central transition (i.e. $+1/2 \rightarrow -1/2$) is observed and the other three spin–spin relaxation components are broadened beyond detectibility. At 7.05 T, the line width is substantially greater than at 11.7 T, and a 5 ppm up-field shift is observed which is attributed to a dynamic frequency shift. The line width of Tf-bound V(V) is not broadened by solvent viscosity up to 50% glycerol/buffer (v/v). Measurements of signal intensity as a function of pulse length reveal increased ^{51}V precession frequencies in the radio frequency field, as frequently observed for studies of half-integer quadrupolar nuclei in the solid state [134].

Two additional pieces of information are available which, if taken together with the previous data, allow us to develop a picture of V(V) coordination to transferrin. As outlined earlier, it has become commonplace to talk about separate anion binding and metal binding sites in transferrin. V(V) binding to transferrin is unique in that it is blocked or inhibited by other metal ions, i.e. Fe^{3+} or Ga^{3+} as well as by anions such as arsenate, phosphate or sulfate [131]. Secondly, in contrast to virtually all other metal ions, V(V) binding to transferrin requires no additional synergistic anion [131]. Thus it appears

that vanadate has binding characteristics both of a metal and of a synergistic anion [131]. Ultraviolet difference spectra of vanadium-transferrin and model compound studies indicate that vanadium(V) is coordinated by two tyrosine residues [131]. Further, the VO_2^+ moiety is the most likely species to be coordinated to transferrin, since other forms of V(V) such as bare V^{5+} or VO^{3+} are excluded by the lack of a discernable LMCT band expected for these ions bound to phenolate ligands [75]. Simple association as the vanadate ion, $H_2VO_4^-/HVO_4^{2-}$ is eliminated by the magnitude of the formation constant and the nature of the UV difference spectrum [131]. A hypothetical vanadium site consistent with the observations could be vanadium(V) coordination by Tyr 426, Tyr 517 and Asp 392 with hydrogen bonding of the V=O oxygens to His 585 and Arg 456 (Fig. 21). The coordination geometry could well be trigonal bipyramidal *or* octahedral if another ligand is coordinated.

To return to the physiological significance of the various species, it is clear that both the vanadyl- and vanadate-transferrin complexes can be stable under physiological conditions. Chasteen et al. have examined the redox interaction between the two species as well as the binding of the vanadyl and vanadate ions with serum albumin, another metal-binding protein [136]. The presence of endogenous reducing agents and dissolved oxygen appears to ensure that both oxidation states coexist in the blood plasma. Evidence is presented that the vanadyl ion will be partitioned between transferrin and the albumin while the vanadium(V) will be bound exclusively to transferrin. Several aspects of the kinetics of vanadium metabolism in blood have also been discussed by Harris et al. [132].

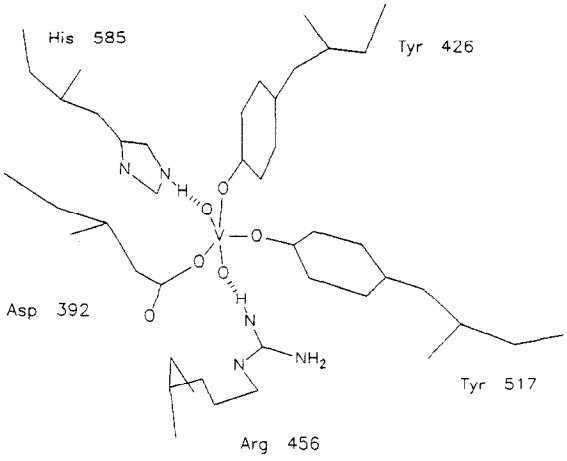


Fig. 21.

(ii) Vanadoenzymes

While vanadium has been shown to be important in biological systems and is, in fact, an indispensable trace element for many organisms, authentic examples of vanadium in the active site of enzymes have been discovered only recently. In 1984, Vilter demonstrated that certain marine algae contain a non-heme bromoperoxidase that is activated by vanadium(V) [137]. A brief review of the history of this discovery is available [138]. In addition to vanadium-bromoperoxidase, a second naturally occurring vanadoenzyme has been discovered, which is a vanadium-containing nitrogenase [139,140]. The vanadium bromoperoxidase and vanadium nitrogenase are described below.

(a) Vanadium bromoperoxidase

Vanadium bromoperoxidase (V-BrPO) has been isolated from several species of marine brown algae, including *Ascophyllum nodosum* [137,141], *Laminaria saccharina* [142], *Fucus distichus* [143], and *Macrocystis pyrifera* [143], the red alga, *Ceramium rubrum* [144], and a terrestrial lichen, *Xanthoria parietina* [145]. In addition, vanadium-dependent bromoperoxidase activity has been detected in many other species of brown macroalgae [141,146]. These enzymes can catalyze the formation of carbon–halogen bonds and thus have been implicated in the biogenesis of the many different brominated natural products found in marine organisms. Many of these brominated compounds seem to have important chemical defense-type functions [147]. Two different vanadium bromoperoxidases, which differ in carbohydrate content, have been isolated from *A. nodosum* [141,148]. The most abundant bromoperoxidase, V-BrPO-I, was found in the thallus and the other bromoperoxidase, V-BrPO-II, was reported to be present on the thallus surface [141]. A previous report also concluded that V-BrPO is present in two different locations of *A. nodosum*, one found in the cell walls of the transitional region between the cortex and medulla of the thallus and the other of which is in the cell wall of the thallus surface [149]. More recent experiments demonstrate that vanadium-dependent bromoperoxidase activity is present in both the cortical and surface protoplasts of *M. pyrifera* [146]. A non-heme iron bromoperoxidase has also been reported in certain species of *Rhodophyta* [150,151], however recent reports showing the presence of a small amount of vanadium in these enzymes has been used to suggest that vanadium may be the active-site metal ion [152,153].

The vanadium-bromoperoxidases are all acidic proteins ($pI \sim 5$) [141] of ca. 65 000 Da per subunit with very similar amino acid compositions [154]. As isolated, these proteins contain only about 0.4 vanadium atoms per subunit, however one gram-atom of vanadium per subunit can be achieved by addition of excess vanadate and subsequent removal of adventitiously

bound vanadium(V) [141,143]. Active-site vanadium(V) has been completely removed by EDTA in phosphate buffer, resulting in concomitant removal of bromoperoxidase activity [137,155]. The activity of the apo derivative is fully restored upon complexation of vanadate, demonstrating that vanadium is essential for catalytic activity [155]. V-BrPO (*A. nodosum*) has been crystallized, although refined structural data have not been completed yet [156].

V-BrPO slowly loses activity in phosphate buffer [148]. Vanadate reconstitution of apo-BrPO is also inhibited in the presence of phosphate buffer, an effect which de Boer and coworkers believe arises from the structural similarities of phosphate and vanadate [154]. Wever et al. state that breakage of vanadium-oxygen bonds upon reincorporation of vanadate is unlikely and conclude that phosphate binds to the enzyme in the same manner as vanadate, i.e., as the oxy-anion coordinated by two additional protein ligands [154]. On the contrary, protonation of vanadium-bound oxo and hydroxo ligands leads to facile water exchange, demonstrating that other ligands can easily coordinate to vanadium(V) [83]. Phosphate is an excellent complexing agent and could sequester bromoperoxidase-bound vanadate, leading to formation of the inactive, apo-BrPO derivative and a polyoxo vanadium-phosphate species [157]. In fact, phosphate-inactivated V-BrPO can be fully reactivated by removal of phosphate followed by addition of vanadate. The isostructural molybdate ion is also capable of blocking reconstitution of the apo-protein by vanadate which has also been used to suggest that vanadate and molybdate compete competitively for the same site [155].

The presence of the pentavalent oxidation state of vanadium in V-BrPO is established by a combination of the (1) lack of an EPR signal in the native enzyme either alone or in the presence of bromide and hydrogen peroxide substrates, (2) appearance of a V(IV) ESR signal upon one-electron reduction of native V-BrPO and (3) reactivation of apo-BrPO by the addition of vanadate ($H_2VO_4^-/HVO_4^{2-}$). Reduction of the native enzyme with dithionite gives rise to a normal anisotropic axial EPR spectrum typical of the binding of the vanadyl ion (d^1) to a protein moiety [155]. Detailed analysis of the EPR spectrum at pH 4.2 reveals $g_{\|} = 1.950$, $g_{\perp} = 1.980$, $g_o = 1.970$, $A_{\|} = 167.5$ G, $A_{\perp} = 55.1$ G, $A_o = 92.6$ G. These parameters are consistent with N,O coordination to vanadium(IV) [155]. EPR spectra recorded in D_2O or $H_2{}^{17}O$ instead of $H_2{}^{16}O$ show changes in line widths consistent with at least one water ligand for the vanadium [155]. The EPR spectrum is also reversibly pH-dependent such that at pH 8.4 new parameters, $g_{\|} = 1.948$, $g_{\perp} = 1.979$, $g_o = 1.969$, $A_{\|} = 160.1$ G, $A_{\perp} = 50.2$ G, and $A_o = 86.8$ G are observed. The pH dependence is attributed to the ionization of a functional group with a pK of 5.3 [155]. Aspartate, glutamate or a perturbed histidine are all candidates for this coordinated ligand. Alternatively, the ionization of vanadium(V)-bound water is also a reasonable possibility since it can be expected that

water bound to a strong Lewis acid like VO_2^+ will undergo hydrolysis in a similar pH regime. Neither peroxide nor halide ions perturb the EPR spectrum, indicating that these substrates bind only to V(V). Of course it must be remembered that the proposed ligation of V(IV)-BrPO inferred from the EPR data may well be expected to be different from that of the native V(V) enzyme due to quite different coordination geometry preferences of the two oxidation states.

Electron spin echo envelope modulation (ESEEM) spectroscopy of the reduced V-BrPO compared with a variety of vanadyl compounds suggests that a nitrogen ligand is present in the equatorial plane of the BrPO-bound vanadyl cation [158]. An intense ^1H modulation was also observed which was replaced by ^2H modulation when VO^{2+}-BrPO was present in D_2O instead of H_2O, further confirming the presence of an exchangeable proton at the vanadium site.

A ^{51}V NMR signal for the native V(V) enzyme has been reported [159]. The NMR spectrum displays a very broad (i.e. 6 kHz) signal at about -1250 ppm. The quality of the data is unfortunately quite poor but the authors suggest that the drastic increase in shielding may indicate some η^2-bound ligands, however the high field shift observed for the ^{51}V signal in the presence of the substrate iodide is opposite to the direction predicted by simple shift-electronegativity considerations.

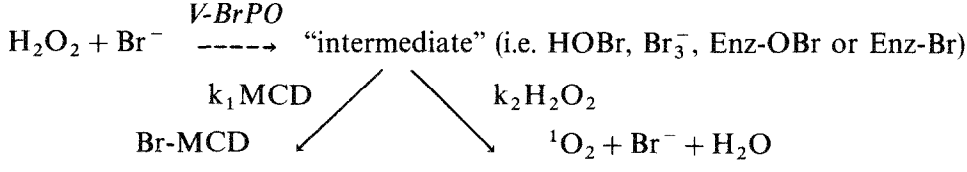
The K-edge and X-ray absorption near-edge structures (XANES) of native V-BrPO in the absence or presence of bromide or hydrogen peroxide are very similar, while the dithionite-reduced V-BrPO is substantially different [160]. A very recent report on the EXAFS structure of V-BrPO also suggests that vanadium(V) is coordinated by ca. three unknown light-atom donors ca. 1.72 Å, 2 nitrogen donors at 2.11 Å, almost certainly histidine nitrogens and a single terminal oxo group at 1.61 Å, all arranged in a distorted octahedral geometry [161]. No observable differences were found in the EXAFS upon treating the enzyme with either, or both hydrogen peroxide or bromide. A significant change was observed on dithionite reduction, consistent with a distorted octahedral coordination sphere for V(IV) with a single vanadyl oxygen at 1.63 Å and 5 N(O) ligands in two groups (i.e. 3 at an average distance of 1.91 Å and 2 at 2.11 Å). The fit was significantly improved by considering second shell scattering as from imidazole carbon atoms, strongly suggesting at least two histidines among the five N(O) donors. The 2.11 Å distance is also consistent with this view. Thus, the fundamental change seen upon reduction is the lengthening of the three other vanadium–light atom bonds from the ca. 1.72 Å seen in the native vanadium(V) enzyme to 1.91 Å in the vanadium(IV) derivative.

The specific activity of V-BrPO is measured spectrophotometrically at 290 nm by the rate of bromination of monochlorodimedone (2-chloro-5,5-

dimethyl-1,3-dimedone, MCD) and is expressed as μmol MCD brominated per min per mg enzyme (i.e. U mg^{-1}). MCD is the classic substrate used to determine the specific activity of haloperoxidases. V-BrPOs have maximum specific activities in the range of 120–1700 U mg^{-1}, depending on pH, bromide and hydrogen peroxide concentrations [143,155].

Bromoperoxidases catalyze the oxidation of bromide by hydrogen peroxide, resulting in the bromination of certain organic substrates or the production of dioxygen in the absence of a suitable organic substrate. V-BrPO is unlike FeHeme haloperoxidases in that significant dioxygen formation occurs *only* in the presence of bromide or iodide, demonstrating the lack of true catalase activity [162], although the stoichiometry of dioxygen formed to hydrogen peroxide consumed is 1/2, indicating the overall reaction is the disproportionation of hydrogen peroxide [163]. The halide-assisted disproportionation of hydrogen peroxide is catalyzed by bromide as demonstrated by bromide-selective electrode techniques [162]. Under conditions when dioxygen formation is completely inhibited during bromination (e.g. 75 μM MCD, 2 mM H_2O_2 at pH 6 for V-BrPO from *A. nodosum*), the rate of MCD bromination is equal to the rate of dioxygen formation in the absence of MCD, but otherwise identical conditions [162,164]. The dioxygen produced is in the singlet excited state ($^1O_2$;$^1\Delta_g$), which was recently shown by analysis of the near infrared emission (i.e. λ_{max} 1268 nm), the near stoichiometric yield of $^1O_2$ and the reduced emission intensity in the presence of specific $^1O_2$ quenchers [148]. The remarkable feature of V-BrPO is its stability in the presence of high concentrations of oxidized bromide species or singlet oxygen. By comparison, the FeHeme haloperoxidases, chloroperoxidase and lactoperoxidase were inactivated by 50 and 100%, respectively, under conditions in which V-BrPO retained full activity [148]. The rate of production of singlet oxygen was equal to the rate of dioxygen formation measured by an oxygen electrode, confirming that the pathways of singlet oxygen formation and dioxygen formation are the same.

The equality of the rates of MCD bromination and dioxygen formation suggests that both reactions proceed through the formation of a common intermediate, the production of which is rate-limiting (Scheme 1 [148,162]). The k_1[MCD] reaction is competitive with the $k_2[H_2O_2]$ reaction [164],



Scheme 1.

since at high concentrations of hydrogen peroxide, the sum of the rate of dioxygen formation during MCD bromination and the rate of MCD bromination is equal to the rate of dioxygen formation in the absence of MCD at the same hydrogen peroxide concentration. Thus the apportionment of the intermediate is fully accounted for by the k_1[MCD] and $k_2[H_2O_2]$ pathways [164].

The exact nature of the intermediate in Scheme 1 has not been identified unambiguously. Reaction of hydrogen peroxide with any of the proposed intermediates is consistent with singlet oxygen production. Singlet oxygen is a well-established product of the reduction of $HOBr/Br_2/Br_3^-$, or bromamines by hydrogen peroxide [165]. Chiral halogenated terpenes are well known natural products in many marine organisms (for a review, see ref. 147), suggesting that a direct enzyme-halogenation reaction occurs as opposed to 'chemical' halogenation by $HOX/X_2/X_3^-$. De Boer and Wever have proposed that Br_3^-/HOBr is the brominating moiety based on the appearance of an absorbance increase at 290 nm (i.e. the λ_{max} of Br_3^- and HOBr) [169]. Their conditions, which include high bromide concentration, *very* high enzyme concentration and low pH (i.e. pH 5), actually favor and possibly force the formation of tribromide. For example, under the same conditions of pH and bromide concentration, *N*-bromosuccinamide reacts with bromide forming tribromide, demonstrating the tremendous ease of displacement of a bromonium ion from a brominated amine. At low pH, the yield of singlet oxygen from $HOBr/Br_3^-$ and hydrogen peroxide is also reduced [165]. Thus, while Br_3^-/HOBr is produced at low pH, this is not necessarily the brominating intermediate under physiological conditions. At neutral pH, tribromide or hypobromous acid cannot be detected because of the very rapid reduction of the oxidized bromine species by hydrogen peroxide.

V-BrPO catalyzes the formation of many other brominated compounds (e.g. phenols, alkenes, pyrimidines, sulfur heterocycles, etc), however, none of these are efficiently brominated since most of the hydrogen peroxide is shunted off in the bromide-assisted catalase reaction [162]. The nature of the peroxide, however, strongly affects the rate of oxygen formation. V-BrPO can use acyl peroxides (e.g. peracetic acid, *m*-chloroperoxybenzoic acid, phenyl peracetic acid) to catalyze bromination reactions, but not alkyl peroxides (e.g. ethyl hydroperoxide, *tert*-butyl hydroperoxide, cuminyl hydroperoxide) [143]. Neither acyl peroxides nor alkyl peroxides catalyze dioxygen formation, either in the presence or absence of a halide [143]. Bromamine formation has been proposed as a possible intermediate for the Fe-Heme haloperoxidases, although it has not been detected [165,166]. Recently, bromamine formation catalyzed by V-BrPO using peracetic acid as the peroxide source was observed for the first time [143]. Primary amines (e.g. Tris) formed the most stable bromamines, although secondary and tertiary amines have also

been identified. Br-Tris rapidly brominates MCD and is reduced by hydrogen peroxide producing dioxygen (presumably $^1O_2$) in reactions that are too fast to be measured by conventional mixing or stopped-flow techniques. These results suggest that bromamines or possibly brominated sulfur compounds could be the active halogenating intermediates of the haloperoxidases [143].

The detailed role of the active-site vanadium ion, i.e. whether it functions as an electron transfer catalyst of bromide oxidation or a Lewis acid catalyst, is not known. The vanadyl-BrPO state is probably not an important component because an EPR signal is not observed during turnover conditions and because VO^{2+}-BrPO does not have bromoperoxidase activity. Most other peroxidases are Fe-Heme-containing systems, the mechanisms of which are reasonably well understood in terms of two-electron oxidation–reduction reactions. Hydrogen peroxide oxidizes the Fe-Heme moiety by two electrons, forming Compound 1 [167]. Compound 1, in turn, reacts with the halide ion to form the active halogenating species. It is clear, however, that such a mechanism cannot be operative in the vanadium(V)-dependent haloperoxidase enzymes of marine algae because the vanadium is already in its highest accessible oxidation state.

Vanadium could function as a Lewis acid catalyst or an electron transfer catalyst of the oxidation of bromide by peroxide. In the Lewis acid scheme (Fig. 22), vanadium(V) coordinates hydrogen peroxide which could then oxidize bromide. Vanadium would not formally change its oxidation state. In an electron transfer scheme (Fig. 23), analogous to the two-electron changes of other peroxidases and avoiding the inactive VO^{2+}-BrPO state, vanadium

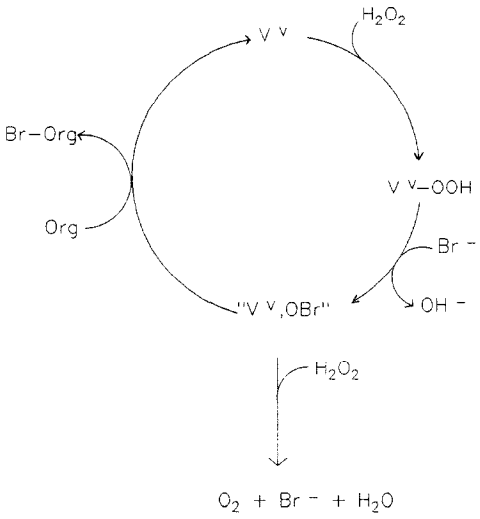


Fig. 22.

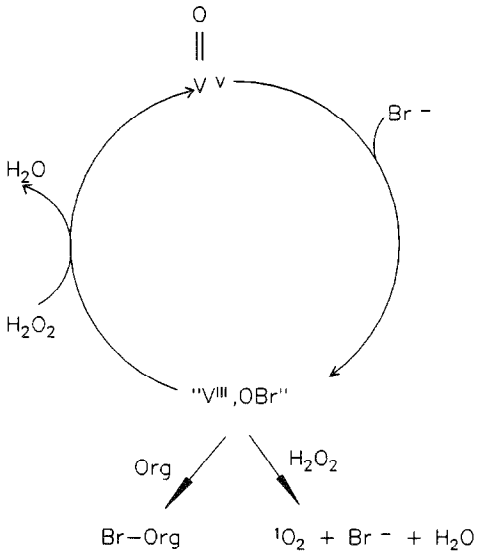
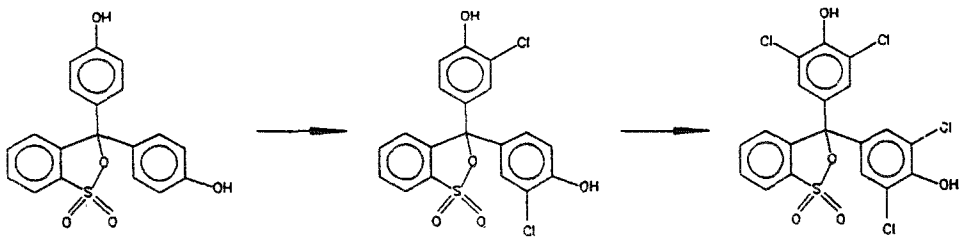


Fig. 23.

could cycle between V(V) and V(III), in which the halide reduces V(V) by two electrons producing V(III). The peroxide would function to oxidize V(III) back to V(V) or reduce the oxidized bromide species forming bromide and singlet oxygen. There is considerable precedence for both types of reaction, as in the two-electron reduction of V(V)-tetraglyme by bromide (electron transfer catalysis) [90] and in the oxygenation of organic substrates by oxygen-atom transfer from V(V)-peroxides (Lewis acid catalysis) [66,70]. Since conventional spectroscopic techniques (e.g. UV–Vis, ^{51}V NMR) cannot be used to observe the active site under turnover, vanadium model complexes will likely prove very useful to probe the function of the vanadium. Preliminary results on the oxidation of iodide by monoperoxovanadium(V) followed by ^{51}V NMR indicates vanadium functions as a Lewis acid catalyst of iodide oxidation by hydrogen peroxide [168].

Detailed steady-state analyses of the rate of MCD bromination [164,169] and dioxygen formation [164] catalyzed by V-BrPO from *A. nodosum* fit a substrate-inhibited bi-bi ping pong kinetic mechanism in which the substrate bromide acts as an inhibitor at certain pH values. The initial kinetic study showed that bromide was a competitive inhibitor of hydrogen peroxide binding to V-BrPO at pH 4–5.5. A subsequent investigation clearly shows a non-competitive contribution [164]. The kinetic parameters (K_m^{Br}, $K_m^{H_2O_2}$, K_i^{Br}) obtained in the dioxygen formation reaction and the MCD bromination reaction agree within a factor of ca. two, providing further evidence that the rate-limiting steps in both reactions are the same [164].

Very recently, V-BrPO has been shown to catalyze chlorination reactions and the chloride-assisted disproportionation of hydrogen peroxide [170]. Chlorination of Phenol Red (i.e. phenolsulfonephthalein) produces dichlorophenolsulfonephthalein and subsequently tetrachlorophenolsulfonephthalein [170].



Chlorination of amines (e.g. taurine, etc.) is also catalyzed by V-BrPO, forming the stable chloramine derivative, even in the presence of hydrogen peroxide. Unlike bromamines, the chloramine is not reduced by hydrogen peroxide. The specific chloroperoxidase activity as measured by MCD chlorination is ca. 1000 times less than the specific bromoperoxidase activity, although unlike the marine Fe-Heme bromoperoxidase (*P. capitatus*) the pH optima of chlorination and bromination are the same. Further experiments are in progress to assess the physiological relevance of the V-BrPO-catalyzed chlorination reactions.

(b) Vanadium nitrogenase

The existence of a vanadium-containing nitrogenase in nitrogen-fixing bacteria was first suspected several decades ago and stemmed from several reports of vanadate-stimulated nitrogen fixation [171]. However, it has only been very recently that a vanadium-nitrogenase has been isolated and purified [172,173]. Vanadium-nitrogenase constitutes an alternative nitrogenase system that is expressed in certain bacteria when they are grown in the presence of vanadium [174].

All conventional nitrogenases are comprised of two distinct proteins, MoFe-protein and Fe-protein. The MoFe-protein, i.e. component 1, which contains the active site for substrate reduction, is tetrameric ($\alpha_2\beta_2$) containing 30–33 iron atoms and two molybdenum atoms. The Fe protein, i.e. component 2, (MW 64 000) contains four iron atoms and four acid labile sulfide ions and functions as a reductant of the MoFe-protein. The alternative nitrogenase system of *Azotobacter vinelandii* and *A. chroococcum* also contain two protein components. Component 1 of the alternative nitrogenase differs from the conventional nitrogenase in the structure of the polypeptide and

the content of metal ions. Hales et al. [172] and Robson et al. [173] have demonstrated that the alternative nitrogenases of *A. vinelandii* (i.e. Av1′) and *A. chroococcum* (i.e. Ac1*), respectively, contain vanadium instead of molybdenum. Component 1 of *A. chroococcum* is a tetrameric ($\alpha_2\beta_2$) protein of 210 000 Da comprised of two different subunits of MW ca. 55 000 and 50 000. Ac1* binds 2 vanadium atoms, 23 iron atoms and 20 acid labile sulfide ions [175]. In addition, Ac1* has an *N*-methylformamide extractable cofactor, FeVco, similar to the FeMo-cofactor of the conventional nitrogenase. Component 1 of the *A. vinelandii* V-nitrogenase (i.e. Av1′) is very similar with a MW of ca. 200 000 Da. and a V/Fe ratio of $1/13 \pm 3$ [172]. The metal content of the Fe protein of Av2′ [172] and Ac2* [176] are essentially identical to the Fe-protein of the conventional nitrogenase, i.e. containing four iron atoms and four acid labile sulfide ions per protein (MW 60 000).

The low-temperature EPR spectra of both dithionite-reduced VFe-nitrogenase proteins (i.e. Av1′ and Ac1*) resemble the reduced MoFe-nitrogenase proteins (i.e. Av1 and Ac1) in that both contain EPR signals indicative of a species with an $S = 3/2$ ground state, however, the V-enzymes differ slightly from each other. The EPR spectrum of reduced Av1′ has g values at 5.80, 5.40, 4.34, 2.04, and 1.93 [173,177]. The g signals at 2.04 and 1.93 comprise a minor component of the total spin (i.e. 0.2–0.3 spin per molecule) and are associated with the $S = 1/2$ state (i.e. the S1 signal). The signal at $g = 4.34$ has been attributed to the presence of adventitious high spin Fe^{3+} ($S = 5/2$). The signals at $g = 5.80$ and 5.40 are associated with the $S = 3/2$ ground state (i.e. the S2 signal). Very recently, a third signal (i.e. the S3 signal) has been found in Av1′ which is indicative of a paramagnetic site coupled to a diamagnetic center [178]. The S2 and S3 signals overlap, prohibiting the determination of the integrated intensity of each signal. S2 and S3 comprise the major spin components while S1 is minor component [178]. The EPR spectrum of reduced Ac1* is characterized by g values of 5.6, 4.35, 3.77 and 1.93 [175]. This last signal is representative of an $S = 1/2$ center, which has been attributed to Fe-S in P-type clusters, while the other signals arise from the $S = 3/2$ ground state associated with the vanadium site. The major difference between the two vanadium-nitrogenases lies in the presence of a $g = 3.77$ signal in the Ac1* spectrum which is absent in the Av1′ spectrum [177]. By comparison, the EPR spectrum of the conventional FeMo nitrogenase is characterized by g values of 4.3, 3.6, and 2.0 associated with the 3/2 ground state of the M-type clusters (slightly rhombic with positive zero-field splitting). The P-type clusters are diamagnetic in the resting state and paramagnetic ($S = 5/2$ established by magnetic susceptibility and Mossbauer spectroscopy) but EPR-silent in the oxidized state. The vanadium nitrogenases have (Av1′) a higher rhombicity and smaller axial zero-field splitting than the conventional Mo-enzymes [177].

The low-temperature magnetic circular dichroism (MCD) spectra also indicate the presence of two new clusters in the VFe nitrogenases [179]. One cluster is paramagnetic with $S=3/2$ ground state in dithionite-reduced Av1′ which becomes diamagnetic upon thionine oxidation. This cluster is attributed to the V-Fe site. The other cluster is paramagnetic in the thionine oxidized state, but diamagnetic in the dithionite reduced state which resembles the P-type cluster of the conventional MoFe protein, although the P-type cluster in the V and Mo proteins may not be identical.

From the vanadium K X-ray absorption edge for the Av1′ vanadium-nitrogenase [180], the vanadium site has been interpreted as resembling the Kovacs and Holm VFe_3S_4 cubane-like cluster [28]. Av1′ contains 3 ± 1 V–Fe interactions at a distance of 2.76 Å, 3–4 V–S interactions at a distance of 2.33 Å and 2–3 V–O (or N) interactions at 2.15 Å. Ac1* is very similar, although the primary difference is that the predicted number of sulfur ligands for Av1′ is higher than that predicted for Ac1*, which is 2 ± 1 [181]. The EXAFS results also show that the primary difference between the vanadium and molybdenum enzymes occurs in the spread between the M–S and M–Fe distances: $\Delta R=0.42$ Å between the V–S and V–Fe distance vs. $\Delta R=0.32$ Å between the Mo–S and Mo–Fe distance.

The reactivity of the vanadium-nitrogenase systems do differ from the conventional nitrogenases. The most distinguishing characteristic is the formation of ethane from acetylene in addition to ethylene, catalyzed by the vanadium enzyme system [182], whereas the molybdenum enzyme system only forms ethylene from acetylene. Ethane formation has been proposed as a discriminating test of the presence of the vanadium enzyme [183]. The alternative systems differ from each other in the relative rates of dinitrogen and acetylene reduction in that dinitrogen is a much better substrate for Av1′ than acetylene by a factor of >4, whereas acetylene is a slightly better substrate than dinitrogen for the conventional nitrogenase ($3N_2/C_2H_2$ 0.78) [172]. By comparison, the ratio of $3N_2/C_2H_2$ for both *A. chroococcum* enzymes is about unity. If the reactivity is referenced to dihydrogen formation, acetylene reduction by Av1′ is poorer than Ac1*, but dinitrogen reduction is more facile.

H. OTHER SYSTEMS

(i) Vanadyl-substituted bleomycin

The bleomycins are a group of glycopeptides that bind to and cleave DNA in the presence of dioxygen (or a reduced oxygen species, e.g. hydrogen peroxide) and a metal ion (e.g. iron) [184]. Vanadyl ion also forms a 1:1, orange complex with bleomycin between pH 6 and 10 [185]. The UV–Vis

spectrum is characterized by absorption maxima at 467 and 758 nm which have been assigned to d_{xy}–$d_{x^2y^2}$ and d_{xy}–$d_{xz,yz}$ ligand field transitions. The EPR spectrum is consistent with square pyramidal coordination of VO^{2+} by five nitrogen ligands, producing an octahedral complex. The proposed ligands include a secondary amine nitrogen, the pyrimidine nitrogen, a deprotonated amide nitrogen, histidyl imidazole nitrogen and an α-amine nitrogen as an axial ligand, consistent with the proposed ligands for other metal bleomycin complexes. Hydrogen peroxide causes the EPR signal to disappear, presumably oxidizing V(IV) to V(V) producing hydroxyl radical, since addition of dithionite restored the original EPR signal. Other examples of vanadium(IV)-induced Fenton-like chemistry are known [186].

VO^{2+}-bleomycin catalyzes double stranded DNA (i.e. supercoiled pBR 322) scission in the presence of excess hydrogen peroxide, forming nicked and linear DNA [185]. Vanadyl-bleomycin is about 50 times less effective than iron-bleomycin under the conditions employed. Nucleotides adjacent to guanine bases such as G-C and G-A (5′→3′) were preferentially cleaved by the vanadium bleomycin derivative. In addition, vanadium-bleomycin attacked G-A (5′→3′) sequences more frequently than the iron-bleomycin complex. The authors suggest that the different reactivities of the vanadyl and ferric bleomycins might be due to different configurations [185].

(ii) Vanadyl-substituted dopamine β-monooxygenase

Dopamine β-monooxygenase is a copper-containing enzyme that catalyzes the hydroxylation of dopamine, forming norepinephrine. Vanadyl has been reported to activate the copper-depleted dopamine β-monooxygenase derivative [187]. The vanadyl derivative contains four equivalents of VO^{2+} per enzyme tetramer. While the vanadyl ion can catalyze the autoxidation of some catecholamines, vanadyl did not catalyze the oxygenation of dopamine under the conditions of the assay. Although vanadyl ion was less effective than Cu^{2+} at reactivating the enzyme (i.e. 300-fold excess required with VO^{2+} versus 10-fold excess with Cu^{2+}), the maximal level of activity produced was exactly the same for both. An EPR spectrum is reported but no parameters are given, leaving questions about the binding site open.

(iii) Vanadocene interaction with nucleic acids

With the discovery in 1969 of the potent antitumor properties of *cis*-diamminedichloro-platinum (II) by Rosenberg and Van Camp [188], interest in transition metal complexes for chemotherapy has soared. Although most of the early work centered on determining structure–activity relationships designed to produce 'second generation' square planar Pt(II) complexes,

recent work has shown that other metals with other geometries also form active complexes [188]. One of the most surprising classes of complexes to show good antitumor activity is the metallocene dihalides or bis-(pseudo-halides) [189]. These complexes of the form Cp_2MX_2 where $Cp = \eta^5\text{-}C_5H_5$, and M = Ti, V, Nb, or Mo, and $X = F^-$, Cl^-, Br^-, I^-, NCS^-, and N_3^- (Fig. 24) have been reported by Kopf-Maier to be highly active against Ehlich ascites, lymphoid leukemia, and lymphocytic leukemia cells. The possibility that the mechanism of action of these complexes might be related to that of the *cis*-platinum is based on the presence of the topologically similar *cis*-MX_2 functionality. Nevertheless solid evidence that supports or denies this contention has been lacking until recently. Since the intracellular target of all these materials is expected to be DNA and the reactions all occur in aqueous solution, a knowledge of how Cp_2VX_2 behaves in aqueous solution with the constituents of the DNA molecule is a prerequisite to understanding their mode of action. Such work has recently been reported by Marks and coworkers [190,191]. These workers have found that, among the series Cp_2MX_2 where M = V, Ti, Cr, only V–Cp bonds are stable to hydrolysis over periods of up to hours making Cp_2VX_2 the compound of choice for further studies. They also found that the V–Cl bonds are hydrolyzed much more rapidly and extensively than the corresponding Pt–Cl in '*cis*-platin'. The resulting aquo complex is also far more acidic than the corresponding Pt-H_2O with pK_a values of 4.73 and 5.15.

Using proton and ^{31}P NMR, EPR as well as X-ray crystallography, Marks and coworkers have found that Cp_2VCl_2 binds preferentially to the phosphate groups of the DNA backbone rather than to the accessible nitrogenous sites on purine and pyrimidine bases [191]. This vanadium–phosphate interaction is very kinetically labile and Watson–Crick base pairing between nucleotides in aqueous solution is not disrupted by the presence of Cp_2VCl_2. These observations stand in sharp contrast to what is seen for '*cis*-platin', where a

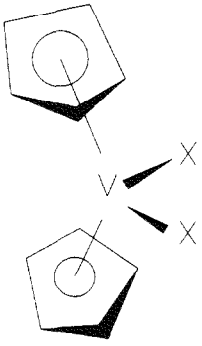


Fig. 24.

kinetically inert covalent bond between Pt and the endocylic nitrogen atoms of the purine or pyrimidine bases (particularly N-7 of guanine) are formed which significantly alter the base pairing in solution between guanine and cytosine. These differences can be accounted for on the basis of 'hard' and 'soft' acid/base considerations. Taken together, the results show that the mechanism of action of Cp_2VCl_2 as an anti-cancer drug is fundamentally different from that of '*cis*-platin' and hence this class of compound should prove to be very interesting for future study.

I. SUMMARY AND A LOOK TO THE FUTURE

The last half decade has witnessed many advances elucidating the biological role of vanadium with the recent discoveries of vanadium bromoperoxidase, vanadium nitrogenase, the isolation and synthesis of tunichrome and the developments in the structural analysis of Amavadin. Several novel coordination compounds of vanadium have also been discovered recently, most notably the many new examples of bare V(IV) and V(V) catecholate and phenolate complexes, the bare V(IV) sepulchrate and hydroxamate complexes, and the vanadium iron sulfur cubane and double cubane clusters. These advances have been fueled by a symbiotic relationship that is developing between the biochemistry and coordination chemistry of vanadium. For example, the VFe_3S_4 clusters were actually synthesized and characterized before vanadium nitrogenase was discovered and have contributed its characterization. The discovery of vanadium bromoperoxidase has reactivated interest in vanadium peroxide complexes and in oxygen-atom transfer reactions of vanadium. One of the more striking developments is the realization that the terminal oxygen of vanadium(IV) and vanadium(V) moieties are far more reactive than previously believed. The terminal oxygen can be removed either by protonation or with strongly oxophilic reagents. The oxygen can also be removed by oxygen-atom transfer, although relatively few examples of this type of reaction are known. Physical techniques to monitor vanadium are also improving, particularly ^{51}V NMR which can be used to monitor V(V), despite a nuclear spin of 7/2, in many different complexes, including large proteins. Many other areas warrant further investigation.

Looking to the future, more examples of vanadium-containing enzymes are likely to be found, such as the possibility of a vanadium-storage protein in the gene-altered *Azotobacter* that produces vanadium nitrogenase, analogous to the molybdenum-storage protein [192]. In recent years, a series of reports has appeared indicating that 'peroxyvanadate' is a potent insulin mimetic agent [193,194]. The mode of action appears to be activation of the insulin receptor kinase via an inhibition of phosphotyrosine phosphatase. The exact nature of the so called 'peroxyvanadate' has not been addressed.

The marine environment would seem to be a likely place to find new vanadium proteins or other biologically occurring vanadium complexes since, after molybdenum, vanadium is the most abundant transition metal ion in ocean water [195]. However, unlike molybdenum, which does not show a 'nutrient-like' distribution profile as a function of ocean depth, the concentration of vanadium is depleted in surface ocean water, indicating that vanadium is a required nutrient for surface-living organisms. Considering the abundance of vanadium in the ocean, it is not surprising that two of the examples of biologically occurring vanadium enzymes or complexes were found in marine organisms. We wait with eager anticipation for the discovery of these new vanadium biomolecules and are confident that the marriage of inorganic chemistry and biochemistry will continue to lead toward a better understanding of the role of vanadium in biology.

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REFERENCES

1 W. Mertz, Science, 213 (1981) 1332.
2 K. Schwartz, Fed. Proc. Fed. Am. Soc. Exp. Biol., 33 (1974) 1748.
3 L.C. Cantley, L. Josephson, R. Warner, M. Yanaqisawa, C. Lechene and G. Guidotti, J. Biol. Chem., 252 (1977) 7421. L. Josephson and L.C. Cantley, Biochemistry, 16 (1977) 4572.
4 T. Kobayashi, T. Martensen, J. Nath and M. Flavin, Biochem. Biophys. Res. Commun., 80 (1978) 1313.
5 L.C. Cantley, Curr. Top. Bioenerg., 11 (1981) 201.
6 D. Boyd and K. Kustin, Adv. Inorg. Biochem., 6 (1985) 311.
7 N.D. Chasteen, Struct. Bonding, 107 (1983) 53.
8 G. Willsky, in N.D. Chasteen (Ed.), Vanadium in Biological Systems, Kluwer, Dordrecht, 1990, pp. 1–24.
9 B.R. Nechay, Annu. Rev. Toxicol., 24 (1984) 501.
10 R.A. Coulombe, D.P. Briskin, R.J. Keller, W.R. Thornley and R.P. Sharma, Arch. Biochem. Biophys., 255 (1987) 267.
11 S. Liochev, E. Ivancheva and I. Fridovich, Arch. Biochem. Biophys., 269 (1989) 188.
12 T. Ramasara and F.L. Crane, Curr. Top. Cell Regul., 20 (1981) 247.
13 D.G. Drueckhammer, J.R. Durrwachter, R.L. Pederson, D.C. Crans and C.-H. Wong, J. Org. Chem., 54 (1989) 70.

14 D.C. Crans, E.M. Willging and S.R. Butler, J. Am. Chem. Soc., 112 (1990) 427.
15 D.C. Crans, C.D. Rither and L.A. Theisen, J. Am. Chem. Soc., 112 (1990) 2901.
16 L.V. Boas and J.C. Pessoa, in G. Wilkinson (Ed.), Comprehensive Coordination Chemistry, The Synthesis, Reactions, Properties and Applications of Coordination Compounds, Vol. 3, Pergamon Press, Oxford, 1987, pp. 453–583.
17 A. Butler, in N.D. Chasteen (Ed.), Vanadium in Biological Systems, Kluwer, Dordrecht, 1990, pp. 25–49.
18 C.R. Randall and W.H. Armstrong, J. Chem. Soc. Chem. Commun., (1988) 986.
19 P.J. Blower, J.R. Dilworth, T. Hutchinson, T. Nicholson and J.A. Zubieta, J. Chem. Soc. Dalton Trans., (1985) 2639.
20 R.W. Wiggins, J.C. Huffman and G. Christou, J. Chem. Soc. Chem. Commun., (1983) 1313.
21 J.R. Rambo, J.C. Huffman and G. Christou, J. Am. Chem. Soc., 111 (1989) 8027.
22 J.K. Money, J.C. Huffman and G. Christou, J. Am. Chem. Soc. 109 (1987) 2210.
23 J.K. Money, K. Folting, J.C. Huffman, D. Collison, J. Temperley, F.E. Mabbs and G. Christou, Inorg. Chem., 25 (1986) 4583.
24 H. Sakurai, Z. Taira and N. Sakai, Inorg. Chem. Acta, 151 (1988) 85.
25 J.C. Dutton, G.D. Fallon and K.S. Murray, Inorg. Chem., 27 (1988) 34.
26 T.R. Halbert, L.L. Hutchings, R. Rhodes and E.I. Stiefel, J. Am. Chem. Soc., 108 (1986) 6437.
27 Y.-P. Zhang and R.H. Holm, Inorg. Chem., 27 (1988) 3875.
28 J.A. Kovacs and R.H. Holm, J. Am. Chem. Soc., 108 (1986) 340.
29 J.A. Kovacs and R.H. Holm, Inorg. Chem., 26 (1987) 702.
30 J.A. Kovacs and R.H. Holm, Inorg. Chem., 26 (1987) 711.
31 S. Ciurli and R.H. Holm, Inorg. Chem., 28 (1989) 1685.
32 P. Comba, L.M. Engelhardt, J.M. Harrowfield, G.A. Lawrence, L.L. Martin and A.M. Sargeson, J. Chem. Soc. Chem. Commun., (1985) 174.
33 C.J. Carrano, unpublished data.
34 E. Kime-Hunt, K. Spartalian, M. DeRusha, C.M. Nunn and C.J. Carrano, Inorg. Chem., 28 (1989) 4392.
35 P. Knopp, K. Wieghardt, B. Nuber, J. Weiss and W.S. Sheldrick, Inorg. Chem., 29 (1990) 363.
36 L.J. Calvion, J.M. Arber, W. Clegg, D. Collison and C.D. Garner, Abstract N026, Fourth International Conference on Bioinorganic Chemistry, Cambridge, MA, July 23–28 1989.
37 J. Bultitude, L.F. Larkworthy, D.C. Povey, G.W. Smith, J.R. Dilworth and G. Leigh, J. Chem. Soc. Chem. Commun., (1986) 1748.
38 R. Cammack, Nature (London), 333 (1988) 595.
39 M. Koppen, G. Fresan, K. Weighardt, R.M. Llusar, B. Nuber and J. Weiss, Inorg. Chem., 27 (1988) 721.
40 P. Reichard and A. Ehrenberg, Science, 221 (1983) 514.
41 R.E. Stenkamp, L.C. Sieker, L.H. Jenson and J. Sanders-Loehr, Nature (London) 291 (1981) 263.
42 W.F. Beyer and I. Fridovich, Biochemistry, 24 (1985) 6460.
43 E. Bayer and H. Kneifel, Z. Naturforsch., 27 (1972) 207.
44 C.G. Trick, Curr. Microbiol., 18 (1989) 375.
45 L.A. Actis, W. Fish, J.H. Crosa, K. Kellerman, S.R. Ellenberger, F.M. Hauser and J. Sanders-Loehr, J. Bacteriol., 167 (1986) 57.
46 R.M. Cassidy and D.E. Ryan, Can. J. Chem., 46 (1968) 327.
47 C.G. Trick, R.J. Andersen, N.M. Price, A. Gillam and P.J. Harrison, Mar. Biol., 75 (1983) 9.
48 M.A.F. Jalal, M.B. Hossain, D. van der Helm, J. Sanders-Loehr, L.A. Actis and J.H. Crosa, J. Am. Chem. Soc., 111 (1989) 292.
49 K. Wieghardt, U. Quilitzch, B. Nuber and J. Weiss, Angew. Chem. Int. Ed. Engl., 17 (1978) 351.
50 K. Wieghardt, W. Holzbach, E. Hofer and J. Weiss, Inorg. Chem., 20 (1981) 343.

51 V. Chilou, P. Gouzerh, Y. Jeannin and F. Robert, Inorg. Chem. Acta., 133 (1987) 205.
52 E. Bayer, E. Koch and G. Anderegg, Angew. Chem. Int. Ed. Engl., 26 (1987) 545.
53 M.A.A.F. de C.T. Carrondo, M.T.L.S. Duarte, J.C. Pessoa, J.A.L. Silva, J.J.R. Frausto da Silva, M.C.T.A. Vaz and L.F. Vilas-Boas, J. Chem. Soc. Chem. Commun., (1988) 1158.
54 K.N. Raymond, G. Muller and B.F. Matzanke, Top. Curr. Chem., 123 (1984) 49.
55 Y.K. Agrawal, G.D. Mehd and H. Environ., Anal. Chem., 10 (1981) 183.
56 K.R. Pande and S.G. Tendon, J. Inorg. Nucl. Chem., 42 (1980) 1509.
57 D.C. Fister, S.J. Barclay-Peet, C.A. Balfe and K.N. Raymond, Inorg. Chem., 28 (1989) 4399.
58 V.L. Pecoraro, Inorg. Chim. Acta., 155 (1989) 171.
59 I. Batinic, M. Birus and M. Pribanic, Croat. Chem. Acta., 60 (1987) 279.
60 S. Luterotti and V. Grdinic, Analyst, 111 (1986) 1163.
61 A. Butler, S. Parsons, S.K. Yamagata and R.I. de la Rosa, Inorg. Chem. Acta., 163 (1989) 1.
62 F. Preuss, W. Towae and J. Woitschach, Z. Naturforsch., 35 (1980) 817.
63 D. Rehder, W. Priebsch and M. von Oeynhausen, Angew. Chem. Int. Ed. Engl., 28 (1989) 1221.
64 H. Sakurai and K. Tsuchiya, FEBS Lett., 260 (1990) 109.
65 R.E. Drew and F.W.B. Einstein, Inorg. Chem., 12 (1973) 829.
66 H. Mimoun, L. Saussine, E. Daire, M. Postel, J. Fischer and R. Weiss, J. Am. Chem. Soc., 105 (1983) 3101.
67 H. Mimoun, P. Chaumette, M. Mignard and L. Saussine, Nouv. J. Chim., 7 (1983) 467.
68 C. Djordjevic, B.C. Puryear, N. Vuletic, C.J. Abelt and S.J. Sheffield, Inorg. Chem., 27 (1988) 2926.
69 K.A. Jorgensen, Chem. Rev., 89 (1989) 431.
70 H. Mimoun, M. Mignard, P. Brechot and L. Saussine, J. Am. Chem. Soc., 108 (1986) 3711.
71 E.S. Gould, R.R. Hiatt and K.C. Irwin, J. Am. Chem. Soc., 90 (1968) 4573.
72 W.C.A. Wilisch, M.J. Scott and W.H. Armstrong, Inorg. Chem., 27 (1988) 4335.
73 S. Gambarotta, F. van Bolhuis and M.Y. Chiang, Inorg. Chem., 26 (1987) 4303.
74 M. Mazzanti, C. Floriani, A. Chiesi-Villa and C. Guastini, J. Chem. Soc. Dalton Trans., (1989) 1793.
75 S.R. Cooper, Y.B. Koh and K.N. Raymond, J. Am. Chem. Soc., 104 (1982) 4088.
76 U. Auerbach, B.S.P.C. Della-Vedova, K. Wieghardt, B. Nuber and J. Weiss, J. Chem. Soc. Chem. Commun., 15 (1990) 1004.
77 E.I. Stiefel, A. Dori and H.B. Gray, J. Am. Chem. Soc., 89 (1967) 3353.
78 T.W. Hambley, C.J. Hawkins and T.A. Kabanos, Inorg. Chem., 26 (1987) 3740.
79 A.A. Diamantis, J.B. Raynor and P.H. Rieger, J. Chem. Soc. Dalton Trans., (1980) 1731.
80 C.J. Hawkins and T.A. Kabanos, Inorg. Chem., 28 (1989) 1084.
81 D.E. Matsubayashi, K. Akiba and T. Tanaka, Inorg. Chem., 27 (1988) 4744.
82 J.H. Welch, R.D. Bereman and P. Singh, Inorg. Chem., 27 (1988) 2862.
83 J.A. Bonadies and C.J. Carrano, J. Am. Chem. Soc., 108 (1986) 4088.
84 J.A. Bonadies, V.L. Pecoraro, W. Butler and C.J. Carrano, Inorg. Chem., 26 (1987) 1218.
85 A. Giacomelli, C. Floriani, A. Ofirde Souza Duarte, A. Chiesi-Villa and C. Guastini, Inorg. Chem. 21 (1982) 3310.
86 T.W. Hambley, C.J. Hawkins and T.A. Kabanos, Inorg. Chem., 26 (1987) 3740.
87 M. Pasquali, F. Marchelti and C. Floriani, Inorg. Chem., 26 (1986) 4358.
88 J.A. Bonadies and C.J. Carrano, Inorg. Chem., 25 (1986) 4358.
89 See C.J. Carrano, C.M. Nunn, R. Quan, J.A. Bonadies and V.L. Pecoraro, Inorg. Chem., 29 (1990) 944 and references cited therein.
90 R. Neumann and I.J. Assael, Am. Chem. Soc., 111 (1989) 8410.
91 D. Rehder, Bull. Mag. Res., 4 (1982) 33.
92 D.C. Crans and P.K. Shin, Inorg. Chem., 27 (1988) 1797. D.C. Crans, R.L. Bunch and L.A. Theosen, J. Am. Chem. Soc., in press.
93 A.S. Tracey and M.J. Gresser, Inorg. Chem., 27 (1988) 1269.

94 A.S. Tracey, M.J. Gresser and B. Galeffi, Inorg. Chem., 27 (1988) 157.
95 R.H. Holm, Chem. Rev., 87 (1987) 1401.
96 Y. Zhang and R.H. Holm, Inorg. Chem., 29 (1990) 911.
97 H. Kneifel and E. Bayer, Angew. Chem. Int. Ed. Engl., 12 (1973) 508.
98 H. Kneifel and E. Bayer, J. Am. Chem. Soc., 108 (1986) 3075.
99 M. Asri Nawi and T.L. Riechel, Inorg. Chim. Acta. 136 (1987) 33.
100 N.W. Lepp, S.C.S. Harrison and B.G. Morrell, Environ. Geochem. Health, 9 (1987) 61.
101 K. Kustin and I.C. McCara, Struct. Bonding, 53 (1983) 139.
102 K. Kustin and I.C. McCara, Comments Inorg. Chem., 2 (1982) 1.
103 M.J. Smith, Experientia, 45 (1989) 452.
104 M. Henze, Hoppe-Seyler's Z. Physiol. Chem., 72 (1911) 215.
105 C.J. Hawkins, G.A. James, D.L. Parry, J.H. Swinehart and A.L. Wood, Comp. Biochem. Physiol. B, 76B (1983) 559.
106 A.L. Dungley, K. Kustin, I.G. Macara, G.C. McLeod and M.F. Roberts, Biochem. Biophys. Acta, 720 (1982) 384.
107 P. Frank, R.M.K. Carlson and K.O. Hodgson, Inorg. Chem., 25 (1986) 470.
108 S.G. Brand, C.J. Hawkins and D.L. Parry, Inorg. Chem., 26 (1987) 629.
109 P. Frank, R.M.K. Carlson and K.O. Hodgson, Inorg. Chem., 27 (1988) 118.
110 I.G. Macara, G.C. McLeod and K. Kustin, Biochem. J., 181 (1989) 457.
111 R.C. Bruening, E.M. Oltz, J. Furukawa, K. Nakanishi and K. Kustin, J. Am. Chem. Soc., 107 (1985) 5298.
112 R.C. Bruening, E.M. Oltz, J. Furukawa, K. Nakanishi and K. Kustin, J. Nat. Prod., 49 (1986) 193.
113 E.M. Olta, R.C. Bruening, M.J. Smith, K. Kustin and K. Nakanishi, J. Am. Chem. Soc., 110 (1988) 6162.
114 S. Lee, K. Kustin, W.E. Robinson, R.B. Frankel and K. Spartalian, J. Inorg. Biochem., 33 (1988) 183.
115 H. Michibata, J. Hirata, M. Uesaka, T. Numakunai and H. Sakurai, J. Exp. Zool., 244 (1987) 33.
116 P. Frank, B. Hedmann, R.M. Carlson, T. Tyson, A.L. Roe and K.O. Hodgsen, Biochemistry, 26 (1987) 4975.
117 (a) E. Kime-Hunt, K. Spartalian and C.J. Carrano, J. Chem. Soc. Chem. Commun. (1988) 1217.
(b) E. Kime-Hunt, S. Holmes, M. Mohan and C.J. Carrano, J. Inorg. Biochem., in press.
118 H. Michibata, T. Miyamato and H. Sakurai, Biochem. Biophys. Res. Commun. 141 (1986) 251.
119 N.D. Chasteen, Adv. Inorg. Biochem., 5 (1983) 201.
120 P. Aisen and I. Listowsky, Annu. Rev. Biochem., 49 (1980) 357.
121 J.H. Brock, in P.M. Harrison (Ed.), Metalloproteins, Verlag Chemie, Weinhein, 1985, p. 183.
122 P. Aisen, A. Liebman and J. Zweier, J. Biol. Chem., 253 (1978) 1930.
123 G.A. Rottman, K. Doi, O. Zak, R. Aasa and P. Aisen, J. Am. Chem. Soc., 111 (1989) 8613.
124 S. Bailey, R.W. Evans, R.C. Garratt, B. Gorinsky, S. Hasnain, C. Burgh, H. Shoti, P.F. Lundley, A. Mydin, R. Sarra and J.L. Watson, Biochemistry, 27 (1988) 5804.
125 B.F. Anderson, H.M. Baker, E.J. Dodson, G.E. Norris, S.V. Rumbull, J.M. Waters and E.N. Baker, Proc. Natl. Acad. Sci. U.S.A., 84 (1987) 1768.
126 G.W. Bates and M.R. Schlabach, J. Biol. Chem., 250 (1975) 2177.
127 E. Sabbioni and E. Marafante, Bioinorg. Chem., 9 (1978) 389.
128 See, for example, L.K. White and N.D. Chasteen, J. Phys. Chem., 83 (1979) 279 and references cited therein.
129 N.D. Chasteen, Biol. Mag. Res., 3 (1981) 53.
130 I. Bertini, G. Canti and C. Luchinat, Inorg. Chem. Acta, 67 (1982) 221.
131 W.R. Harris and C.J. Carrano, J. Inorg. Biochem., 22 (1984) 201.
132 W.R. Harris, S.B. Friedman and D.S. Silberman, J. Inorg. Biochem., 20 (1984) 157.
133 A. Butler, M.J. Danzitz and H. Eckert, J. Am. Chem. Soc., 109 (1987) 1864.

134 A. Butler and H. Eckert, J. Am. Chem. Soc., 111 (1989) 2802.
135 P. Holt and A. Butler, work in progress.
136 N.D. Chasteen, J.K. Grady and C.E. Holloway, Inorg. Chem., 25 (1986) 2754.
137 H. Vilter, Phytochemistry, 23 (1984) 1387.
138 R. Wever, E. de Boer, H. Plat and B.E. Krenn, FEBS Lett., 216 (1987) 1.
139 J.E. Morningstar, M.K. Johnson, E.E. Case and B.J. Hales, Biochemistry, 26 (1987) 1795.
140 R.R. Eady, R.L. Robson, T.H. Richardson, R.W. Miller and M. Hawkins, Biochem. J., 244 (1987) 197.
141 B.E. Krenn, M.G.M. Tromp and R. Wever, J. Biol. Chem., 264 (1989) 19287.
142 E. de Boer, M.G.M. Tromp, H. Plat, B.E. Krenn and R. Wever, Biochim. Biophys. Acta, 827 (1986) 104.
143 H.S. Soedjak and A. Butler, Biochemistry, 29 (1990) 7974.
144 B.E. Krenn, H. Plat and R. Wever, Biochim. Biophys. Acta, 912 (1987) 287.
145 H. Plat, B.E. Krenn and R. Wever, Biochem. J., 248 (1987) 277.
146 A. Butler, H.S. Soedjak, M. Polne-Fuller, A. Gibor, C. Boyen and B. Kloareq, J. Phycol. (1990) 589.
147 See S.L. Neidleman and J. Geigert, Biohalogenation, Ellis Horwood, West Sussex, U.K., 1986.
148 R.R. Everett, J.R. Kanofsky and A. Butler, J. Biol. Chem., 265 (1990) 4908.
149 H. Vilter and K.-W. Glombitza, Bot. Mar., XXVI (1983) 341.
150 H. Yamada, N. Itoh and S. Murakami, Agric. Biol. Chem., 49 (1985) 2961. N. Itoh, Y. Izumi and H. Yamada, J. Biol. Chem., 261 (1986) 5194.
151 N. Itoh, Y. Izumi and H. Yamada, J. Biol. Chem., 262 (1987) 11982.
152 H. Yu and J.W. Whittaker, Biochem. Biophys. Res. Commun., 160 (1989) 87.
153 B.E. Krenn, Y. Izumi, H. Yamada and R. Wever, Biochim. Biophys. Acta, 998 (1989) 63.
154 R. Wever, B.E. Krenn, E. de Boer, H. Offenberg and H. Plat, Oxidases and Related Redox Systems, Liss, New York, 1988, pp. 477–493.
155 E. de Boer, K. Boon and R. Wever, Biochemistry, 27 (1988) 1629.
156 A. Muller-Fahrnow, W. Hinrichs, W. Saenger and H. Vilter, FEBS Lett., 239 (1988) 292.
157 M.J. Gresser, A.S. Tracey and K.M. Parkinson, J. Am. Chem. Soc., 108 (1986) 6229.
158 E. de Boer, C.P. Keljzers, A.A.K. Klaassen, E.J. Reijerse, D. Collison, C.D. Garner and R. Wever, FEBS Lett., 235 (1988) 93.
159 H. Vilter and D. Rehder, Inorg. Chem. Acta., 136 (1987) L7.
160 J. Hornes, V. Kuetgens, R. Chauvistre, W. Schreiber, N. Anders, H. Vilter, D. Rehder and C. Weidemann, Biochem. Biophys. Acta, 956 (1988) 293.
161 J.M. Arber, E. de Boer, C.D. Garner, S.S. Hasnain and R. Wever, Biochemistry, 28 (1989) 7968.
162 R.R. Everett and A. Butler, Inorg. Chem., 28 (1989) 393.
163 H.S. Soedjak and A. Butler, unpublished results.
164 R.R. Everett, H.S. Soedjak and A. Butler, J. Biol. Chem., 265 (1990) 15671.
165 J.R. Kanofsky, Arch. Biochem. Biophys., 274 (1989) 229.
166 M. Nieder and L. Hager, Arch. Biochem. Biophys., 240 (1985) 121.
167 See, for example, J.A. Manthey and L.P. Hager, J. Biol. Chem., 260 (1985) 9654 and references cited therein.
168 R.I. de la Rosa and A. Butler, work in progress.
169 E. de Boer and R. Wever, J. Biol. Chem., 263 (1988) 12326.
170 H.S. Soedjak and A. Butler, Inorg. Chem., 29 (1990) 5015.
171 C.E. McKenna, J.R. Benemann and T.G. Traylor, Biochem. Biophys. Res. Commun., 41 (1970) 1501. R.C. Burns, W.H. Fuchmann and R.W.F. Hardy, Biochem. Biophys. Res. Commun., 42 (1971) 353. H.H. Nagatani and W.J. Brill, Biochim. Biophys. Acta, 362 (1974) 160. J.R. Benemann, C.E. McKenna, R.F. Lie, T.G. Traylor and M.D. Kamen, Biochim. Biophys. Acta, 264 (1972) 25.
172 B.J. Hales, E.E. Case, J.E. Morningstar, M.F. Dzeda and L.A. Mauterer, Biochemistry, 25 (1986) 7251.

173 R.L. Robson, R.R. Eady, T.H. Richardson, R.W. Miller, M. Hawkins and J.R. Postgate, Nature (London), 332 (1986) 388.
174 P.E. Bishop, Trends Biochem. Sci. (1986) 225.
175 R.R. Eady, R.L. Robson, T.H. Richardson, R.W. Miller and M. Hawkins, Biochem. J., 244 (1987) 197.
176 R.R. Eady, T.H. Richardson, R.W. Miller, M. Hawkins and D.L. Lowe, Biochem J., 245 (1988) 189.
177 J.E. Morningstar and B.J. Hales, J. Am. Chem. Soc., 109 (1987) 6854.
178 B.J. Hales, A.E. True and B.M. Hoffman, J. Am. Chem. Soc., 111 (1989) 8519.
179 J.E. Morningstar, M.K. Johnson, E.C. Case and B.J. Hales, Biochemistry, 26 (1987) 1795.
180 G.N. George, C.L. Coyle, B.J. Hales and S.P. Cramer, J. Am. Chem. Soc., 110 (1988) 4057.
181 J.M. Arber, B.R. Dobson, R.R. Eady, P. Stevens, S.S. Hasnain, C.D. Garner and B.E. Smith, Nature (London), 325 372.
182 M.J. Dilworth, R.R. Eady and M.E. Eldridge, Biochem. J., 249 (1988) 745.
183 M.J. Dilworth, R.R. Eady, R.L. Robson and R.W. Miller, Nature (London), 327 (1989) 167.
184 J. Stubbe and J.W. Kozarich, Chem. Rev., 87 (1987) 1107.
185 J. Kuwahara, T. Suzuki and Y. Sugiura, Biochem. Biophys. Res. Commun., 129 (1985) 368.
186 H.A. Charmichael, FEBS. Lett., 261 (1990) 165.
187 K.A. Markossian, N.A. Paitian and R.M. Nalbandyan, FEBS Lett., 238 (1988) 401.
188 See, for example, S.J. Lippard (Ed.), Platinum, Gold and Other Metal Chemotherapeutic Agents, ACS Symp. Ser., Vol. 209, 1983.
189 P. Kopf-Maier and H. Kopf, Chem. Rev., 87 (1987) 1137.
190 J.H. Toney and T.J. Marks, J. Am. Chem. Soc., 107 (1985) 947.
191 J.H. Toney, C.P. Brock and T.J. Marks, J. Am. Chem. Soc., 108 (1986) 7263.
192 P.T. Pienkos and W.J. Brill, J. Bacteriol., 145 (1981) 743.
193 S. Kadota, I.G. Fantus, G. Beragon, H.J. Guyda, B. Hersh and B.I. Posner, Biochem. Biophys. Res. Commun., 147 (1987) 259.
194 I.G. Fantus, S. Kadota, G. Deragon, B. Foster and B.I. Posner, Biochemistry, 28 (1989) 8864.
195 K.W. Bruland, in J.P. Riley and R. Chester (Eds.), Chemical Oceanography, Vol. 8, Academic Press, New York, 1983, pp. 157–220.