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The coordination chemistry of vanadium as related to its biological functions

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Contents

Abstract

The binding of vanadium to protein side-chains such as provided by tyrosinate, serinate, aspartate, glutamate, cysteinate, methionine and histidine is modelled by complexes with ligand sets containing phenolate, alkoxide, carboxylate, thiolate, thioether and enamine functions. The complexes mimic possible intermediates and structural motifs of the coordination environment of vanadium in vanadium–nitrogenase, vanadate-dependent haloperoxidases, the interaction with phosphorylation enzymes, and the redox-interaction with cysteinyl residues. The solid state investigations are supplemented by solution studies on vanadate–dipeptide and vanadate–adenosine systems, based on combined ^{51}V-NMR and potentiometric measurements. Model reactions for the function of haloperoxidases and vanadium–nitrogenase (including the alkyne–reductase and isonitrile–reductase/ligase activities) are described, and the impact of these investigations for corresponding in vitro

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applications is dealt with. The relevance of catecholatovanadium complexes for the accumulation of vanadium by ascidians is also addressed.

Keywords: Vanadium; ^{51}V-NMR; Vanadium–nitrogenase; Haloperoxidases; Ascidiaceae

1. Introduction

Two classes of vanadium enzymes, viz. vanadium–nitrogenases and vanadate-dependent haloperoxidases, have so far been found in nature, and their structures and properties have initiated a lightning increase in investigations into vanadium model compounds during the last decade. In vanadium–nitrogenases, vanadium is in a low to medium oxidation state as an integral part of a iron–sulphur cluster [1], which activates and reductively protonates various unsaturated substrates, among these dinitrogen, which is converted to ammonia and hence to a form accessible by plants [2]. The vanadium centre presumably is octahedrally coordinated. In the haloperoxidases, which catalyse the oxidation of halides, vanadium is in the $+$V state in an environment dominated by oxygen functions. Its coordination geometry changes between trigonal-bipyramidal and tetragonal-pyramidal [3,4]. Compounds designed to model the active centre of these haloperoxidases have been reported to also catalyse in vitro oxidations of substrates such as thioethers [5], emphasising the importance of biologically oriented model investigations for industrially relevant processes. Vanadate-dependent haloperoxidases have also been shown to attain phosphatase activity, and this finding may have some impact to medical applications [6]. Another important impetus to vanadium coordination chemistry in the context of medical applications has arisen from the observation that vanadate, peroxovanadate, vanadyl and several vanadium complexes (such as formed with maltol [7]) exert an insulin-mimetic effect [8].

This points to a possibly general role of vanadium in all living organisms: the structural analogy between vanadate ($H_2VO_4^-$ under physiological conditions) and phosphate (HPO_4^{2-}) on the one hand, and the redox-activity of vanadium on the other hand. Vanadate is a known inhibitor (and sometimes stimulator) for many phosphate-metabolising enzymes [9]. This includes the inhibition of a regulatory protein phosphatase which is likely to lead to activation of a protein kinase, the activity of which is key to the insulin-mimetic action of vanadate. The ease by which vanadium changes between the oxidation states $+$V, $+$IV (and $+$III) implements a potentially important role in redox-interaction with cysteine-containing enzymes and other redox-active organics. The redox potential for the pair $VO^{2+} + 3H_2O \rightleftarrows H_2VO_4^- + e^- + 4H^+$ at pH 7 and a ratio $V^V/V^{IV} = 10^3$ amounts to -0.17 V, which is in-between the redox potentials for H_2O/O_2 ($+0.82$) and NADH/NAD$^+$ (-0.32 V). Hence, V^V is usually the stable oxidation state under aerobic conditions, while V^{IV} is present in the reducing intracellular medium and may further be reduced to V^{III}. It is noteworthy that V^V has also been shown to act as an electron acceptor and hence initiator in the photo-cleavage of DNA [10].

Apart from the general role of vanadium in phosphorylation and redox processes, and its specific occurrence in the two enzymes, vanadium has also been found accumulated in sea squirts and mushrooms. Several species of sea squirts (Ascidiaceae) sequester vanadium, mainly in the form of V^{III}, in specialised blood cells [11]. Tunichromes, ascidian pigments which contain catecholate moieties, may be responsible for the reduction of vanadium in the process of uptake of vanadate(V) from sea water to its storage as vanadium(III) [12]. The fly agaric toad stool and other members of the genus *Amanita* contain a low molecular mass vanadium(IV) compound, amavadin, with carboxylate and hydroxylamide ligands coordinated to vanadium [13].

In order to elucidate the broad range of effects vanadium attains in living organisms, we have directed our investigations into the coordination chemistry of vanadium towards systems which model (i) the vanadium binding sites in enzymes, (ii) the geometrical and electronic aspects of vanadium coordination to biogenic molecules, and (iii) the function of vanadium in its active domains. This report will present solid state and solution studies on the structure of vanadium coordination compounds, including—in the context of vanadium–nitrogenase—a few aspects related to organovanadium chemistry. A number of functional studies will also be presented, and emphasis will be placed on those which are of relevance for in vitro applications. Although the author will concentrate on the work done in this area by his group in Hamburg, important contributions from other groups will, of course, be included. Some of the work, such as the solution studies on vanadate–peptide interaction, has been carried out in co-operation with the groups of Prof. Debbie Crans (Fort Collins) and Prof. Lage Pettersson (Umeå).

2. Thio-functional ligands: vanadium–nitrogenase and vanadium–thiolate/thioether interactions

Thio-ligation to vanadium has been reported for vanadium–nitrogenase and for a tyrosyl phosphatase. In the former, vanadium is linked to three sulphides; its oxidation state is between II and IV, and it probably is involved in the electron shuttle accompanying the reduction of N_2, acetylene and other unsaturated compounds [1,2]. In vanadate-reconstituted tyrosyl phosphatase, vanadium(V), in an overall trigonal-bipyramidal environment, is coordinated to an axial cysteinate [14]. Other enzymes with cysteine in their active site, such as glyceraldehyde 3-phosphate dehydrogenase, are redox-inactivated by vanadate [15], suggesting ligation of the cysteinate residue to vanadium prior to the oxidation to disulphide. This kind of interaction is also important when vanadate(V) is reduced to VO^{2+} by glutathione in the intracellular medium. The reduction of the phosphate analogue $H_2VO_4^-$ is, to a certain extent, also a detoxification.

Although so far there are no examples for an interaction between cationic vanadium and naturally occurring organic sulphides, the model compounds described below, and the recent discovery and structural characterisation of a molybdenum enzyme that interacts with dimethyl sulphide, suggest a possible role

for vanadium in this respect. The diagonal relationship between Mo and V, established for a substantial range of the chemistry of these two elements, and also reflected in the analogy of the Mo– and V–nitrogenases, corroborates this assumption.

Vanadium– (or alternative) nitrogenase from the nitrogen fixing bacterium *Azotobacter* contains vanadium instead of the more common molybdenum as an integral part of the *M*-cluster of the iron–vanadium protein. In analogy to its Mo counter-part, the vanadium centre is believed to be bridge-linked by three S^{2-} to three iron centres, and further coordinated to an imidazolyl nitrogen of histidine and the vicinal carboxylate and alkoxo groups of homocitrate (Fig. 1).

Coordination of sulphur-containing ligands has been documented for vanadium in the oxidation states II–V. Fig. 2 shows a selection. Except of the anion **1**, where exclusively sulphur ligands are present, supporting ligands in addition to the thiolate and/or thioether functions constitute the coordination sphere. Thiolate coordination as in **2a**, **4**, **5** and **6–8** is more commonly observed than thioether coordination, which is realised in the V^{II} complexes **3**, **6** and **8**. Interesting cases with respect to the interaction of V^{V} with proteins are the complexes **4** and **2a**. In **4**, the thiolate is constituent of a tridentate ligand reminiscent of a dipeptide. In addition to the thiolate, the carboxylate and the deprotonated amide nitrogen of the glycine moiety are bound to the high valent vanadium centre, a coordination mode also established for dipeptides from solution studies (vide infra). Amide coordination is also evident for **7**, where one of the amino groups of the tetradentate ligand $^{-}SNNS^{-}$ is deprotonated in each half of the dinuclear complex. Complex **2a** can be oxidised with H_2O_2 to the disulphenate complex **2b**.

Some of the compounds displayed in Fig. 2 exhibit redox activity and thus are of particular interest in terms of their model character for vanadium–nitrogenases and redox interaction with cysteine containing peptides and proteins. Thus, the V^{II} complex **8** can reversibly be oxidised to its V^{III} analogue at -0.37 V (vs. SCE). Moreover, the reaction between $[VCl_2(tmeda)_2]$ (tmeda = $Me_2NCH_2CH_2NMe_2$) and $^{-}SS'S'S^{-}$ (cf. Fig. 3, top) does not only yield compound **8**. Concomitantly, reductive elimination of ethylene leads to the formation of a linear trinuclear, mixed-valence $V^{II}(V^{V})_2$ complex **9** with two thiocatecholate ligands on each of the terminal vanadium ions [22]. While, in this case, V^{II} acts as a reducing agent for the tetradentate dithiolate-disulphide $^{-}SS'S'S^{-}$, the formation of **7** from $[VOCl_2(thf)_2]$ and tetradentate dithiolate-diamine $^{-}SNNS^{-}$ is accompanied by an oxidation of

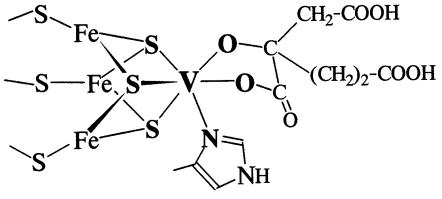


Fig. 1. Proposed vanadium environment in vanadium–nitrogenase, based on XAS findings and analogy to molybdenum–nitrogenases [1,2].

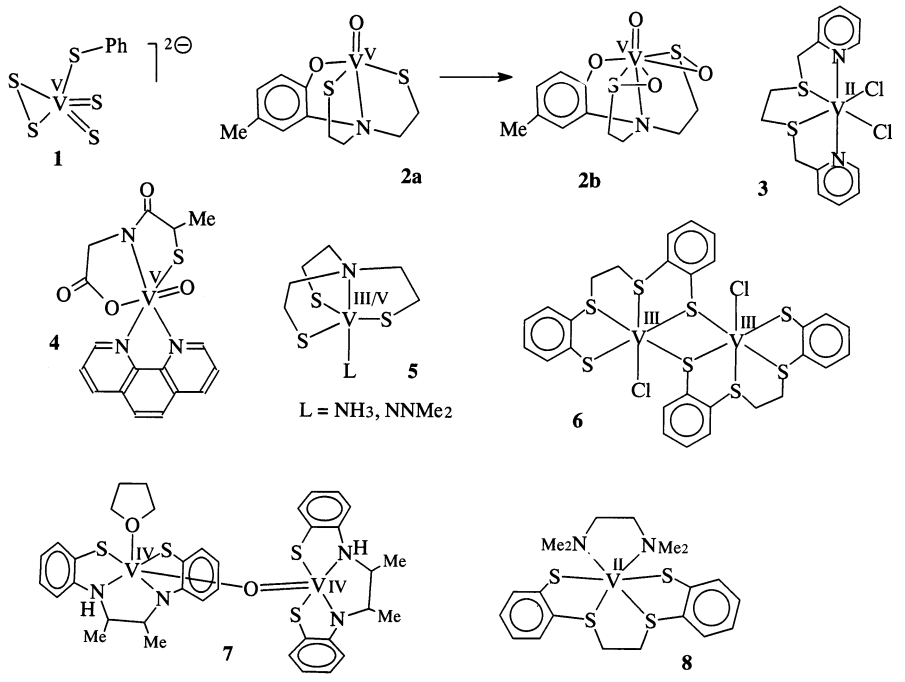


Fig. 2. Structurally characterised vanadium compounds containing thio-functional ligands. **1** [16], **2** [17], **3** [18], **4** [19], **5** [20], **6** and **7** [21], **8** [22].

the ligand to the cyclic bis(disulphide) **10a** (Fig. 3, centre) [21]. Hence, three parallel processes can be observed in this reaction: first, part of the vanadyl precursor compound looses its oxo function and thiolate is oxidised to disulphide. Second, an amide function is formed by deprotonation; and third, the trianionic ligand thus generated coordinates to vanadium. Complex **7** exhibits an additional interesting feature, depicted in Fig. 4, viz. a hydrogen-bond (2.671 Å) between the amine hydrogen in one half of the molecule and the thiolate sulphur in the other half. If the reaction between $[VOCl_2(thf)_2]$ and $^{-}SNNS^{-}$ is carried out with a slight variation in the diamine back-bone of the tetradentate ligand, the cycloeicosane **10b** is obtained together with the tetranuclear oxo-bridged V^{IV} complex **11 (Fig. 3**, centre) [23].

When dealing with V^{V} and V^{IV} complexes, one usually encounters terminal or bridging oxo groups, i.e. VO^{n+} $(n = 3,\ 2)$. This is also so in the case of the compounds **7**, **9** and **11**. A non-oxo vanadium(IV) complex, compound **13**, is obtained from the reaction shown in Fig. 3, bottom: As $[VOCl_2(thf)_2]$ reacts with *o*-mercaptoaniline, part of the ligand is oxidised to the disulphide **12**. Vanadium retains its oxidation state IV by means of an external oxidising agent; the intermediate that is formed is stabilised in a condensation reaction with *o*-hydroxynaphthaldehyde, forming the Schiff base complex **13**. The coordination of this

trifunctional Schiff base ligands apparently prevents it from isomerising to a thiazoline, a conversion which is observed in the absence of a stabilising coordination centre. In **13**, vanadium is in a highly distorted trigonal prismatic environment. The twist angle between the two trigonal planes spanned by S, N and O amounts to 69°; the two planes are inclined towards each other by 28.6° [18].

The interaction between vanadium centres and thio compounds described here does have implications for the application of vanadium catalyst systems in the oxidation of organic sulphides. Thioethers are oxidised by organic and inorganic peroxides to sulphoxides, a process which is sped up by vanadium compounds and directed towards enantio-selectivity, if the catalyst system contains a centre of chirality [5,24,25]. An example is shown in Fig. 5. The catalyst systems may simply consist of a vanadium component such as $VO(acac)_2$ [acac = acetylacetonate(1 −)], an amine and an aldehyde. We have shown that, in such a system, the active intermediate is a Schiff base complex of vanadium such as compound **14** shown in

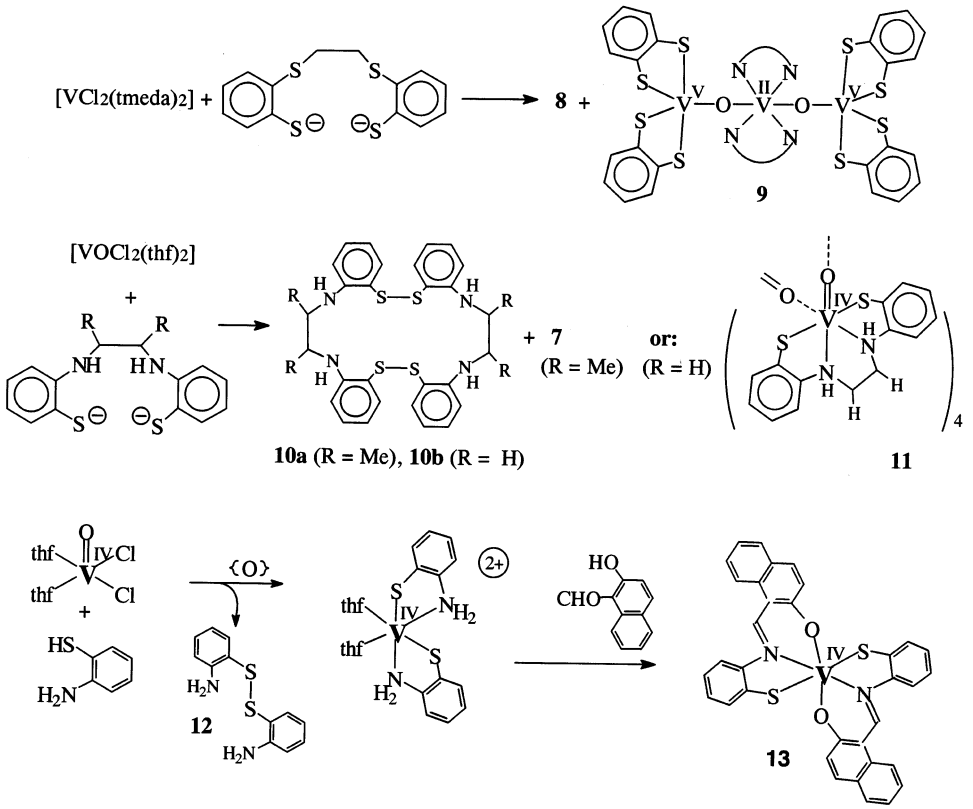


Fig. 3. Redox reactions between vanadium compounds and tetradentate thiophenolate ligands. For compounds **8** and **7** see Fig. 2. References: Top [21], centre [21,23], bottom [18]. A more detailed view of **7** is represented in Fig. 4.

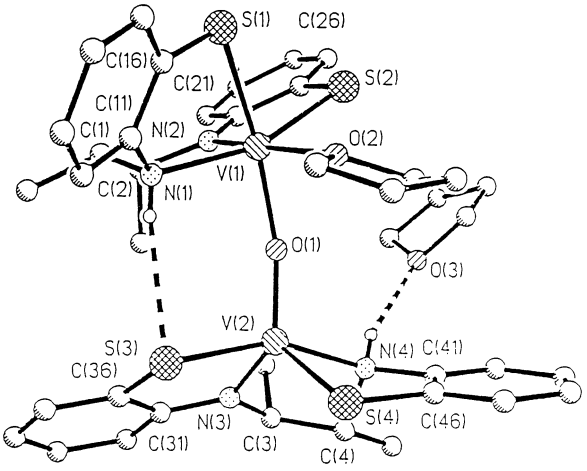


Fig. 4. Molecular structure of **7**·THF, depicting the hydrogen-bonding interactions (broken lines) [21].

Fig. 5, and that these complexes directly interact with the substrate sulphide,possibly by replacing the amine nitrogen which forms a long and hence labile V···N bond of about 2.5 Å [5]. It is of interest to note that the enzyme dmso-reductase, which contains Mo in the co-factor, catalyses the reverse process, i.e. the conversion of dimethylsulphoxide to dimethylsulphide [26], which is one of the important processes in the global sulphur cycle.

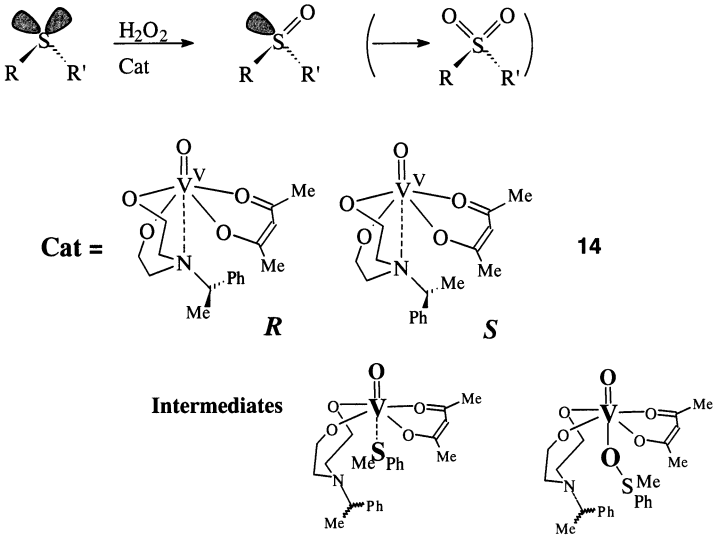


Fig. 5. Oxidation of organic sulphides as catalysed by a V^V complex containing a chiral amino-bis-(ethanolate) [5,31]. Broken lines indicate weak bonds.

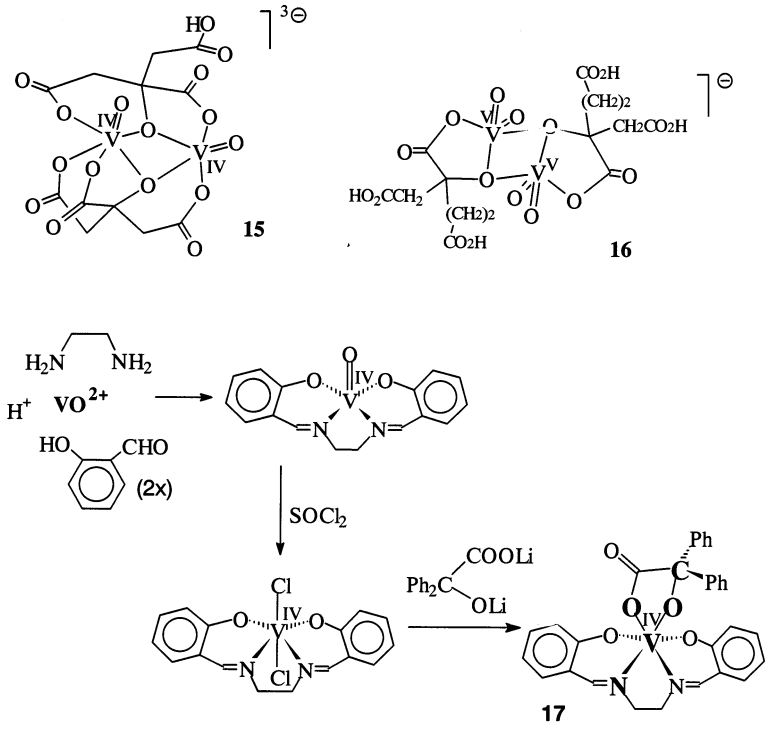


Fig. 6. Complexes which model homocitrate coordination at the vanadium site in nitrogenase. **15** [28], **16** [29], **17** [27].

3. Further aspects related to vanadium–nitrogenases

As has been noted in Fig. 1, homocitrate, binding through vicinal carboxylate plus alkoxide, is an integral part in the coordination sphere of vanadium. Although a few vanadium complexes of citrate and homocitrate are known (e.g. **15** and **16** in Fig. 6), none of these meets the requirements for a satisfying structural model, since they are binuclear complexes of oxo- or dioxovanadium and contain the alkoxide in a bridging mode. A closer model is the benzilate complex **17** in Fig. 6, which lacks the oxo group, contains the correct carboxylate/alkoxide moiety, and also models histidine binding by the enamine nitrogen of the Schiff base. **17** is capable of both, a reversible oxidation at $+0.92$ V and a reversible reduction at -0.34 V (vs. SCE). The synthesis of **17** [27] as depicted in Fig. 6 summarises a general theme allowing access to this type of vanadium complexes: a vanadyl Schiff base complex, in this case the well known [VO(salen)], is generated in a template type of synthesis from the three precursor components. The oxovanadium complex is converted to the dichloro complex *trans*-[VCl_2(salen)], which, via salt metathesis with dilithium benzilate, yields **17**. A variant of this synthesis, the generation of the vanadyl-bis(aldehyde) complex prior to its succeeding reaction with the amine component is

preferred whenever the latter is sterically demanding. In the case of tridentate Schiff bases H*ONO*H, such as those formed from salicylaldehyde and amino acids, complexes of composition $[VO(H_2O)ONO]$ are obtained which, on treatment with thionyl- or acetylchlorides, form the reactive intermediates *cis*-$[VCl_2(ONO)]$ [30–32]. Examples will be discussed in the context of models of haloperoxidases in Section 4.

The primary function of vanadium–nitrogenase is the conversion of N_2 to NH_4^+. An equivalent amount of H_2 and some $N_2H_5^+$ are also formed. Neglecting the latter, the overall reaction is represented by Eq. (1). The formation of a small amount of hydrazine suggest that this compound is an intermediate in the formation of ammonia. This assumption is corroborated by model studies. Thus, treatment of the dinitrogenvanadate($-$I) **18** (Fig. 7) with H^+ yields NH_4^+ and about 1% of $N_2H_5^+$ (Eq. (2)) [33]. Cluster compounds containing the $\{VFe_3S_4\}$ core [34,35] such as $[(dmf)_3VFe_3S_4Cl_3]^-$ have been shown to catalyses the conversion of hydrazine to ammonia and are active in the reduction of phenylhydrazine to aniline plus ammonia [35]. A phenylhydrazide(1$-$) derivative, the cluster **19** in Fig. 7, has been characterised. There are several additional examples, some of which are depicted in Fig. 7, which clearly indicate that hydrazine, hydrazide, diazenide and ammonia can be bound to and activated by vanadium centres.

$$N_2 + 14H^+ + 12e^- + 24ATP \rightarrow 2NH_4^+ + 3H_2 + 24ADP + 24P_i \quad (1)$$

$$\{V^{-I}(N_2)_2\} + 4H^+ \rightarrow NH_4^+ + 1.5N_2 + \{V^{II}\} \quad (2)$$

Complex **18** and related dinitrogen complexes are obtained by reduction of $[VCl_3(thf)_3]$ with sodium or lithium in the presence of phosphines, N_2 and preferably catalytic amounts of naphthalene. V^{II} and mixed $V^{II/I}$ phosphine complexes are formed as intermediates, some of which coordinate hydrazines [39]. The mixed-valence compound $[V_2Cl_3(bdmpm)]$ [bdmpm = $MeP(CH_2CH_2CH_2PMe_2)_2$] reacts with lithiumhydrazide to give ammonia [33]. The dinitrogenvanadate anions attain the

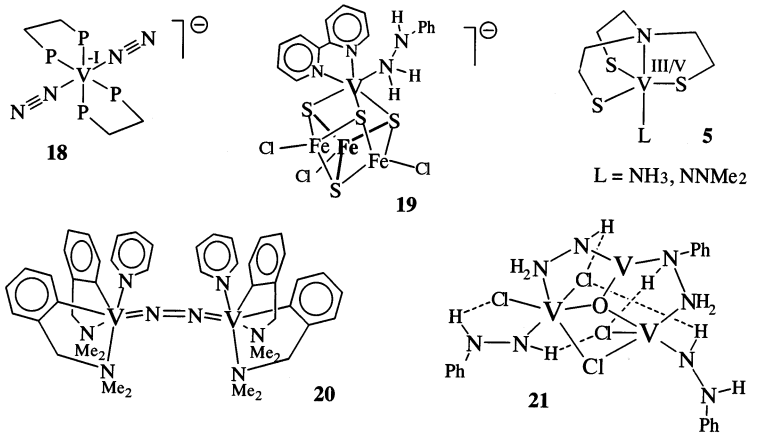


Fig. 7. Nitrogen activation and intermediates in N_2 fixation. **18** [33], **19** [35], **5** [20], **20** [36,37], **21** [38].

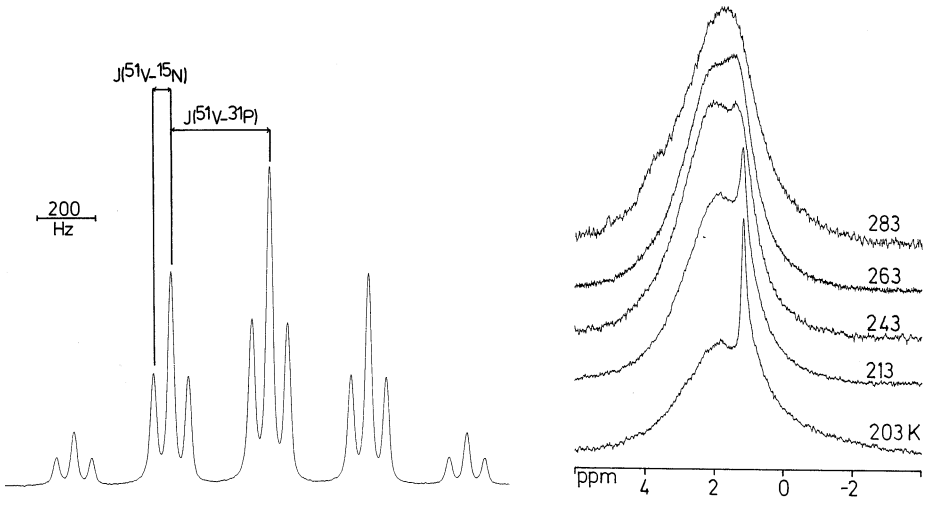
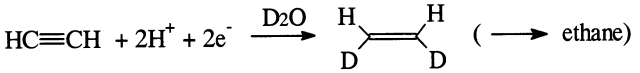


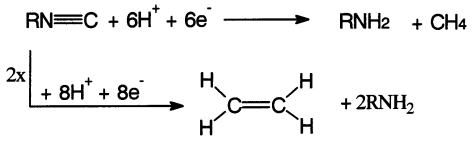
Fig. 8. Left: ^{51}V-NMR spectrum of *trans*-[Na(thf)$_x$][V(^{15}N$_2$)$_2$(dmpe)$_2$] in THF [41]. $\delta(^{51}V) = -1138$ ppm relative $VOCl_3$. Scalar coupling constants (in Hz) are indicated. Right: variable temperature ^{7}Li-NMR spectra of [Li(thf)$_x$][V(N$_2$)$_m$(dmpe)$_n$] + LiCl dissolved in THF [33]. The sharp signal at low temperatures belongs to [Li(thf)$_y$]Cl, the broad one to the lithiumvanadate. At ambient temperature, exchange equilibration prevails.

same molecular structure in solution (cf. the ^{51}V-NMR spectrum in Fig. 8) as in the solid state; they form close contact ion-pairs with solvated Na^+ or Li^+ both in the solid state and in solution, as verified by the X-ray diffraction analysis of [Na(thf)][V(N$_2$)$_2$(dppe)$_2$] (dppe = $Ph_2PCH_2CH_2PPh_2$) [40] and ^{7}Li-NMR spectroscopy of [Li(thf)$_x$][V(N$_2$)$_m$(dmpe)$_n$] (dmpe = $Me_2PCH_2CH_2PMe_2$) [33] (Fig. 8). These contact ion-pairs are essential for their stability.

Apart from reducing protons and dinitrogen, several other unsaturated substrates are also reductively protonated by vanadium nitrogenase. Among these are acetylene and methylisonitrile. Acetylene is reduced to ethylene (and, in part, further to ethane). If this reaction is carried out in D_2O, the *Z* isomer of ethylene is recovered (Eq. (3)), a convincing piece of evidence that the alkyne is activated by coordination to the metal, thus pre-determining the steric conditions prior to reduction and protonation. Methylisonitrile is reduced to methane plus methylamine. In a side-reaction, concomitant C–C coupling occurs, and ethylene is obtained (Eq. (4)), possibly via an intermediately formed acetylene.

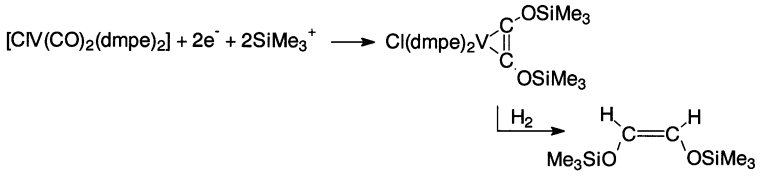


(3)



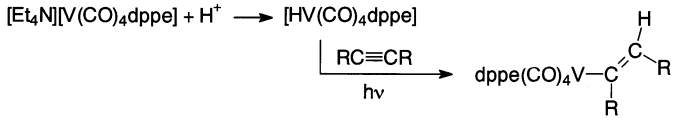
(4)

We have carried out a number of model studies which show that alkynes and isonitriles coordinate to vanadium, forming comparatively stable compounds such as **22** and **23** in Fig. 9. A related isonitrile–niobium(I) complex, **24**, can be converted to the alkyne complex **25**, if protons are provided by simply adding some water. The two electrons necessary for this coupling reaction are provided by the metal, which turns to the +III oxidation state. In a similar manner, metal-mediated carbonyl coupling can be achieved at a vanadium centre, and the resulting alkyne be reduced to the *Z*-alkene by hydrogen (Eq. (5)) [45].



(5)

Vanadium (and niobium) catalyst systems of composition $MCl_n/Zn/H^+$ are widely used in the in vitro reductive protonation and coupling of unsaturated organic substrates ([46]a). A low to medium valent metal centre is likely to be the active species in these reactions, underlining the comparability of the in vitro and in vivo action of vanadium. The metal-mediated reductive protonation of alkynes to alkenes (and of alkenes to alkanes) possibly proceeds through a σ-alkenyl (σ-alkyl) complex. A model reaction for the conversion of an alkyne to a *Z*-(σ-alkenyl) complex is represented by Eq. (6) ([46]b).



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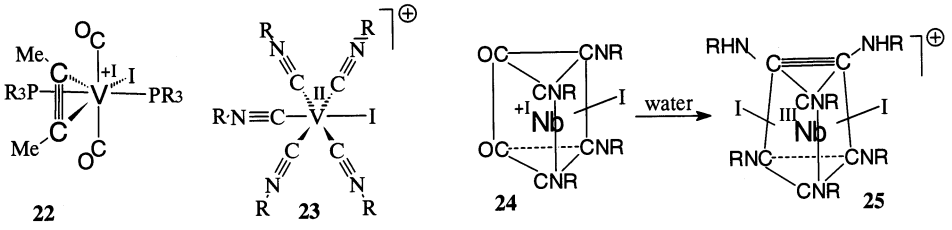

Fig. 9. Models for the binding of alkynes and isonitriles to the active site metal in nitrogenases. **22** [42], **23** [43], **24** and **25** [44].

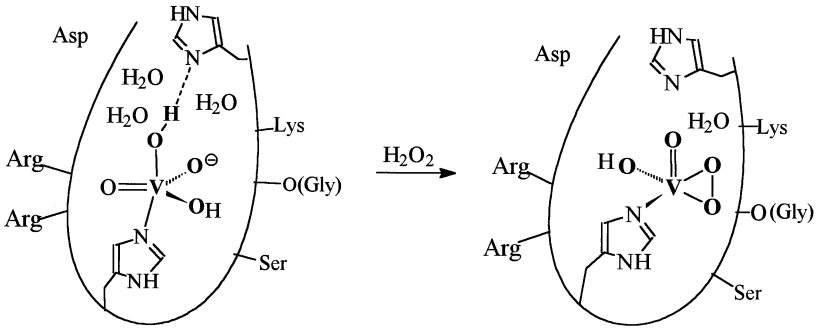

Fig. 10. Active site structures of chloroperoxidase from the fungus *C. inaequalis* according to Ref. [3]. Water molecules and amino acids in H-bonding contacts with the vanadium site are indicated.

4. Implications from studies on structural models of vanadate-dependent haloperoxidases

Vanadate-dependent haloperoxidases contain V^V in their active site. Vanadium can be removed by dialysis against EDTA in citrate buffer, and the activity of the enzyme reconstituted with vanadate. Reduction with dithionite leads to the inactive VO^{2+} form, accompanied by a change in the vanadium environment [47,48]. Exposed to air, the V^V form and the activity are recovered. Reconstitution with VO^{2+} cannot be achieved [49]. The apo-enzyme, when treated with phosphate, exhibits phosphatase activity [6], suggesting interesting evolutionary relationships between peroxidases and phosphatases.

Vanadate-dependent peroxidases have been found in marine brown and red algae, in a lichen and a mould. The latter, a chloroperoxidase isolated from *Curvularia inaequalis*, has recently been characterised by X-ray diffraction both in its native and its peroxo forms [3,4]. Schematic structures of the vanadium sites are shown in Fig. 10. More extensively investigated with respect to the function is a bromo/iodoperoxidase from the sea weed *Ascophyllum nodosum* [50]. First structural results on the *A. nodosum* enzyme have indicated the same vanadium coordination environment as in the *C. curvularia* enzyme. The enzymes catalyse the two-electron oxidation of halide (X^-) by peroxide; X_2, X_3^- or, most likely, hypohalous acid HXO is formed, which halogenates organic substrates RH or, if RH is absent, produces singlet oxygen. A peroxovanadium complex presumably is the active intermediate, as inferred from the structure results on the peroxo enzyme and by model studies [51]. The reactions are summarised in Eq. (7). It should be noted that, despite of the ease of change between the +IV and +V states otherwise characteristic of vanadium, the metal in this case acts as a Lewis acid and not as a redox-active centre.

$$\{VO^{3+}\} + H_2O_2 \rightarrow \{VO(O_2)^+\} + 2H^+$$

$$\{VO(O_2)^+\} + X^- + 3H^+ \rightarrow \{VO^{3+}\} + HXO + H_2O$$

$$HXO + RH \rightarrow RX + H_2O \text{ [or: } HXO + H_2O_2 \rightarrow {}^1O_2 + H_2O + HX] \quad (7)$$

In the native peroxidases, vanadium is in a trigonal-bipyramidal environment (cf. Fig. 10). The vanadate moiety is covalently coordinated to a histidyl side-chain through the imidazol N^{ε}, which occupies an axial position opposite an OH group in hydrogen-bonding contact with water molecules and an additional distal histidine. The structure is very much the same as that of a vanadate reconstituted rat acid phosphatase (where again a histidine is in coordination distance to vanadium [52]), and a low molecular weight bovine heart phosphotyrosyl phosphatase complexed with vanadate, where the axial position is occupied by cysteinate [14]. Addition of peroxide converts the arrangement from trigonal-bipyramidal to tetragonal-pyramidal. The doubly bonded oxygen now is in the apex, while the peroxo ligand stands in the tetragonal plane.

A collection of structural models for the peroxidases is presented in Fig. 11. The selection has been carried out so as to demonstrate the versatility with respect to coordination number, coordination geometry and coordinating functions. All compounds have in common that their coordination sphere is dominated by oxygen functions, one to two of which are oxo groups. Compounds **26** and **27** contain a N_2O_4 donor set with the non-oxo oxygens stemming from alkoxide plus phenolate (**26**), and carboxylate plus water (**27**). In **27**, one of the nitrogens is constituent of

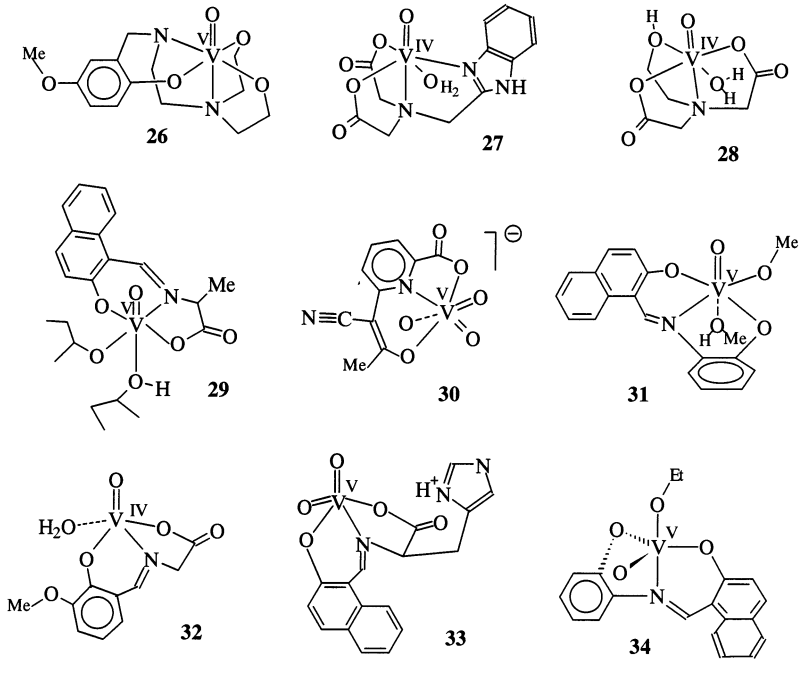

Fig. 11. Structural models for the active site of vanadate-dependent haloperoxidases, and for vanadium binding to tyrosinate, serinate, glutamate and aspartate. **26** [53], **27** [54], **28** [55], **29** [56], **30** ([57]a) **31**, **32** and **34** [31], **33** ([57]b).

an imidazol system and hence models the coordination of histidine in the enzyme. Compounds **28**–**34** contain a NO_{4-5} donor set. The non-oxo functions are carboxylate (**28**, **30**, **32** and **33**), alcohol and alcoholate (**28**, **29**, **31**), phenolate (**29**, **31**–**33** and **41**) or enolate (**30**), the nitrogen functions amine (**28**), pyridine (**29**, **30**) or enamine (**31**–**34**). In some of the complexes with an $NO_{4/5}$ donor set, one of the vanadium-to-oxygen linkages is a weak bond, represented by ligation of water (**28**, **32**), alcohol (**29**, **31**) or μ-O^{2-} (**30**). These bonds may easily be ruptured to provide an additional coordination site for a substrate in a catalytically conducted reaction. This report has already pointed to a comparable case in the context of sulphide oxidation catalysed by complex **14** (Fig. 5). The configurational diversity for complexes with an NO_4 donor set is apparent from comparison of compounds **33** and **34**, the structures of which may be compared with the active sites of chloroperoxidase (Fig. 10): **34** is close to trigonal bipyramidal with the enamine nitrogen *trans* to ethoxide, while **33** is tetragonal-bipyramidal with the enamine-N *cis* to the oxo groups.

The structural motifs depicted in Fig. 11 are also of interest for the (unspecific) interaction between V^{V} or V^{IV} and oxo-functional side chains in peptides and proteins, since they model coordination to tyrosine (phenolate), serine (alkoxide) and glutamate or aspartate (carboxylate). Further, penta-coordinated V^{V} compounds, mainly in the trigonal-bipyramidal geometry, may be considered to model the transition state in phosphoester cleavage, e.g. in the course of hydrolysis of RNA. This report will return to these additional aspects in connection with the solution studies dealt with in Section 6.

Apart from covalent bonds, hydrogen bonds and salt-bridges (contact ion-pairs) are important interlinkages in biological systems, including the participation of metal containing moieties. This situation as represented by the vanadate-dependent haloperoxidases is indicated rather schematically in Fig. 10. Two examples on the basis of the model compounds **33** and **30** in Fig. 11 are depicted in more detail in Fig. 12: compound **33**, *exo*-[VO_2nap-his(H^+)], where nap-his is the dianion of the Schiff base formed from 2-hydroxy-naphthaldehyde and L-histidine, gives rise to an intermolecular hydrogen bonding network. N^{δ} (= N16) of the imidazol is protonated. This proton, the hydrogen of the secondary amine of imidazol (H18), the equatorial oxo group (O4) and the two carboxylate oxygens (O2 and O3) are involved ([57]b). The anions **30**, $[VO_2L]^-$, with L^{2-} a cyano-enolate derivative of picolinate, are linked to chains by bridging oxo groups, and the chains are interlinked by $[Na(MeOH)_2]^+$ cations, with the sodium ions in intimate contact with the coordinated carboxylate oxygens (O2) of one of the $[VO_2L]^-$ chains, and the nitrile nitrogen (N2) of the other chain ([57]a).

While, in $\{[Na(MeOH)_2]^+$ **30**$\}_\infty$, there are covalent bonds between cation and anion, merely electrostatic forces (plus a single weak hydrogen bond) prevail in the sandwich-like compound formed between dihydrogendecavanadate(4 −) and two diprotonated cryptands C221, **34** in Fig. 12. Like monovanadate, decavanadate can inhibit several phosphate-metabolising enzymes. Among these are kinases such as phosphofructokinase ([58]a) and a serine/threonine protein kinase, for which decavanadate serves as a competitive inhibitor of the substrate Leu-Arg-Arg-Ala-Ser-

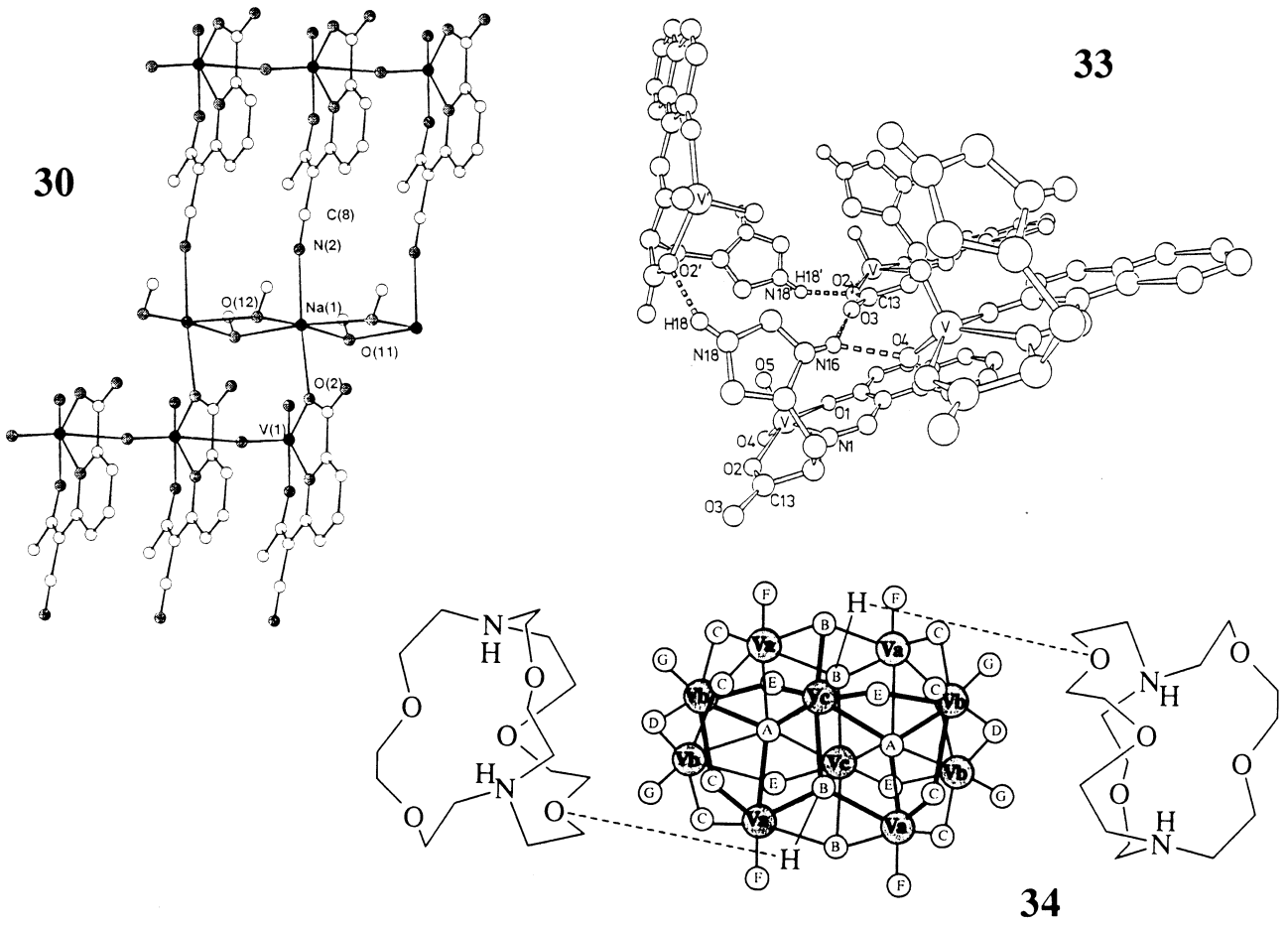

Fig. 12. Sections from the hydrogen bonding network of complex **33**, and the interaction between the anion **30** and its counter-ion $[Na(MeOH)_2]^+$. In **30**, doubly hatched circles correspond to O and N, full circles to V and Na. See Fig. 11 for the molecular structures of **30** and **33**. In $[C221(H^+)_2]_2[H_2V_{10}O_{28}]$ (**34**), cations and anion are kept together by Coulomb forces and weak (3.01 Å) hydrogen bonds (dashed). Protonation site in the anion are the B type (trebly bridging) oxygens [59].

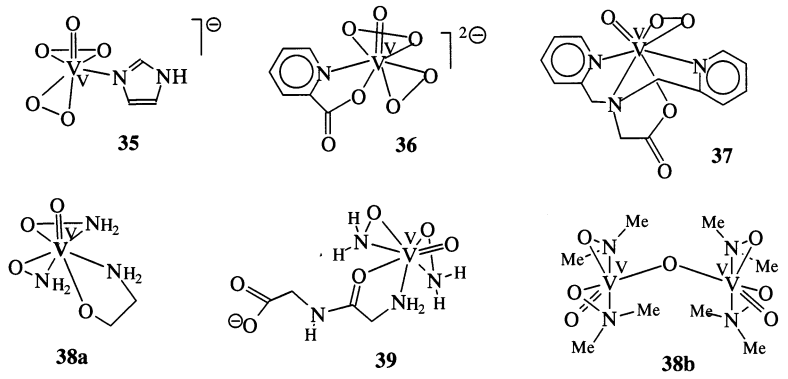

Fig. 13. Peroxo and hydroxylamido complexes of vanadium. **35** [60], **36** [61], **37** [51], **38a** [62], **38b** [64], **39** [62,63].

Leu-Gly (kemptide), probably by binding to the arginine residues ([58]b). Under physiological conditions, however, decavanadate degrades to monovanadate and undergoes, in the intracellular medium, reduction to VO^{2+} ([58]c). Degradation and reduction may be prevented by protection of decavanadate in a manner similar to what is represented by complex **34** [59], where biogenic macrocyclic ligands (ionophores) or oligopeptides such as kemptide (see above) take over the role of the cryptands. Decavanadate is a member of the large group of polyoxometalates, some of which are well known catalysts in organic oxidation chemistry. A reaction similar to that represented for the peroxidase activity in Eq. (7) can be conducted with the Keggin molecule $[H_5PMo_{10}V_2O_{40}]$ ([65]a).

5. Peroxo and hydroxylamido complexes of vanadium

As has been pointed out in the previous section, peroxovanadium complexes very probably are intermediates in halide oxidation by vanadate-dependent peroxidases. They are also potential intermediates in the in vitro oxidation reactions of various organic substrates catalysed by vanadium catalyst systems in oxidations carried out with oxygen or peroxides ([65]b). An example for the latter, the formation of sulphoxides from sulphides, has been set out in Section 2 (see Fig. 5). Further, the insulin-mimetic behaviour of peroxovanadium complexes and their ability to oxidise cysteine have been noted [61]. The $V(O_2)^{n+}$ moiety also plays a role as an intermediate in NADH oxidation as catalysed by V^{IV} [66], and in V^{V}-induced photo-cleavage of DNA [10]. On the other hand, hydroxylamide(1 −), which is isoelectronic with peroxide(2 −), has been found to be a constituent of a ligand system that coordinates non-oxo vanadium(IV) in the biogenic vanadium compound amavadin $[VL_2]^{2-}$ isolated from the mushroom *Amanita muscaria* [13]. The ligand L^{3-} in amavadin is the bis(propionate) derivative of hydroxylamide.

Structures of model complexes are collated in Fig. 13. The anionic bis(peroxo) complex **35** is another (cf. also **27** in Fig. 11) example of the still small number of imidazol–vanadium complexes and hence of complexes which model the histidine binding established for peroxidases. The peroxo complex **37** has been shown to catalyse the two-electron oxidation of bromide and iodide by peroxide in acidic solutions [51]. **36**, in tests with rats, exhibits an insulin-mimetic effect which has been traced back to the oxidation of cysteine to cystine in the cysteine-rich α-subunit of the membrane insulin receptor [61]. Compound **39**, which contains the dipeptide glycyl-glycinate, will be discussed in more detail in the next section.

6. The interaction of vanadate and vanadyl with peptides and nucleosides.

Sections 2–4 had chiefly been dedicated to model compounds for immobilised vanadium at specific proteins sites. Unspecific binding to proteins has, however also been noted [67,68], and many of the model complexes provided in Figs. 2, 6, 11 and 13 may be considered to model vanadium coordination to protein residues containing alcoholic (serine, threonine), phenolic (tyrosine, dopa), carboxylic (aspartic and glutamic acid), nitrogen (histidine, proline, tryptophane) or thio functions (cystein, methionine). A closer approach to model systems for the protein–vanadium interaction is provided by investigation of the coordination properties of protein fragments, i.e. peptides.

As already mentioned, vanadate interacts with a variety of phosphorylation enzymes. Among these are ribonucleases, which are effectively inhibited by vanadate. Ribonucleases catalyse the hydrolysis of a phospho–ester bond. The inhibition may be traced back to an association (via hydrogen bonds [69] or covalent bonds [70]) of vanadate to the active site of the enzyme, thus making it inaccessible for the substrate RNA. Alternatively, vanadate may interact with RNA directly via hydroxyl groups of the nucleotides provided by the phosphate [71] and the ribose moieties (vide infra). Again, model studies on a low molecular weight basis, i.e. studies of the interaction between vanadate and nucleosides should provide insight into the mode of coordination.

One of the advantages of investigating low molecular weight systems is the fact that these systems are easily soluble under physiological conditions, thus providing sufficiently high vanadium concentrations and allowing solution studies through spectroscopic and electrochemical means which otherwise would be hampered due to low concentrations and/or immobilisation. Both, peptides and nucleosides, are present in the intra- and extracellular media and may be the primary ligands to interact with vanadate and vanadyl. To investigate the electronic and steric nature of these primary addition products, as well as the kinetics of their formation and their thermodynamical parameters, is hence an important goal also in understanding the incorporation of vanadium species into macromolecular compounds.

^{51}V-NMR spectroscopy, in combination with potentiometric (emf) studies and NMR of the common nuclei, has turned out to be an adequate means to investigate the vanadate–peptide and vanadate–nucleoside systems [72–79]. Figs. 14 and 15

illustrate the potential of this combination of methods for the system vanadate + L-alanyl-L-histidine [72]. Fig. 14 shows two series of ^{51}V-NMR spectra. In the top series, *c*(vanadate) and pH have been kept constant, and *c*(peptide) varies; in the bottom series, *c*(vanadate) and *c*(peptide) are constant, and the pH varies. There are four rather sharp signals at high field, denoted V_n, belonging to free vanadates which co-exist under these conditions. The V_n are monovanadate ($H_2VO_4^-/HVO_4^{2-}$), divanadate ($H_2V_2O_7^{2-}/HV_2O_7^{3-}$), tetravanadate ($V_4O_{10}^{4-}$) and pentavanadate ($V_5O_{12}^{5-}$), all of which contain vanadium in a tetrahedral environment, where quadrupole relaxation is minimised and hence narrow signals arise. Only mono- (V_1) and divanadate (V_2) are subject to protonation/deprotonation equilibria, and therefore only these signals move as the pH changes. At low field, an additional, broad signal arises, the intensity of which increases as the peptide concentration increases. This signals belongs to a vanadate–peptide complex of the coordination number 5 or 6. ^{1}H-, ^{13}C- and ^{15}N-NMR investigations have revealed the coordinat-

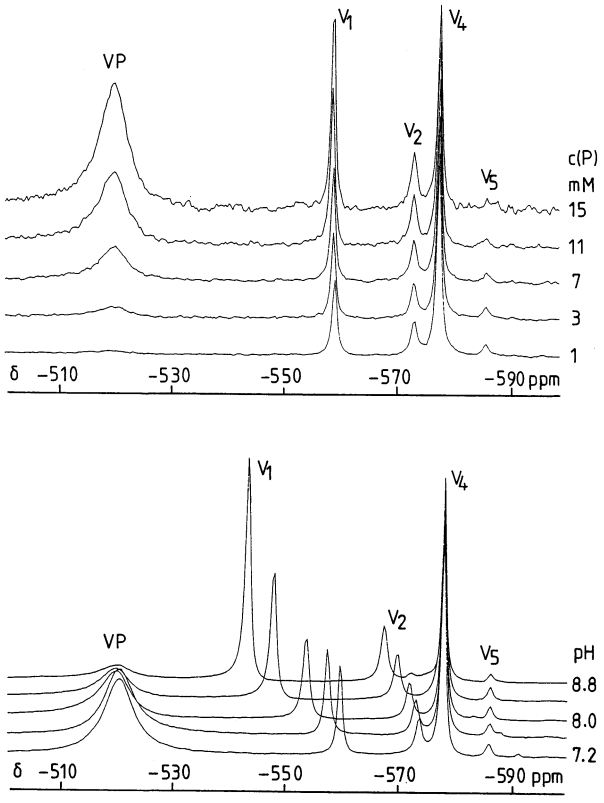

Fig. 14. 94.73 MHz ^{51}V-NMR spectra of the system vanadate/L-alanyl-L-histidine [73]. Chemical shifts are given relative to $VOCl_3$ ($\delta = 0$). The V_n indicate free vanadates (see text), VP is the signal for the monovanadate–peptide complexes **41** in Fig. 16. Ionic strength = 0.6 M (NaCl), *c*(vanadate) = 3 mM; *c*(peptide) = 15 mM in the bottom spectra, pH 7.2 in the spectra on top.

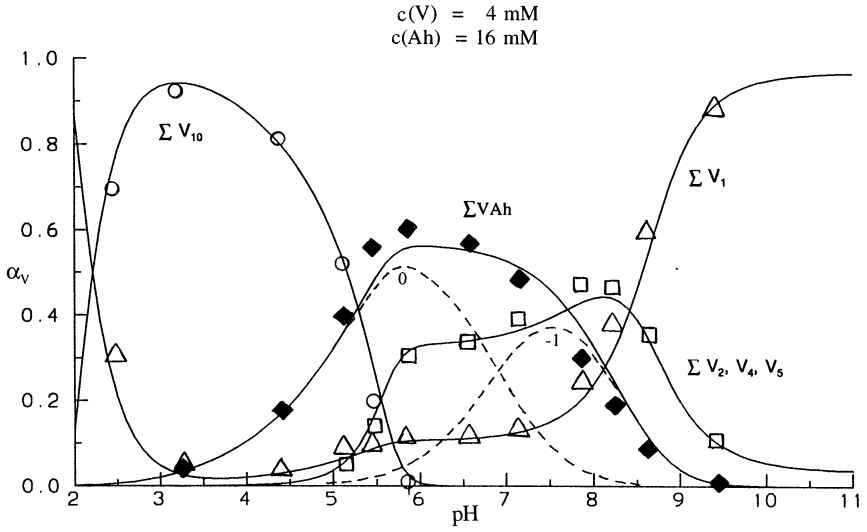

Fig. 15. Diagram showing the distribution of vanadium α(V) versus pH in the vanadate (4 mM)/L-alanyl-L-histidine (16 mM) system at an ionic strength of 0.6 M (NaCl). ΣVAh (◆) is the sum of the vanadate–peptide complexes **41a** and **41b** (cf. Fig. 16). The dashed lines show the distributions of anionic **41a** (− 1; formation optimum at pH 7.5) and neutral **41b** (0; formation optimum at pH 5.8). For V_{1-5} see text; V_1 in the strongly acidic region is VO_2^+. ΣV_{10} (○) is the sum of the decavanadates $H_xV_{10}O_{28}^{(6-x)-}$, $x = 1-3$. The diagram has been provided by Prof. L. Pettersson, Umeå Universitetet.

ing functions of vanadate–dipeptide complexes, viz. the terminal NH_2, the terminal carboxylate and the deprotonated peptide-N [72–74,76]. This tridentate coordination mode involving deprotonated amide has recently been verified for the solid state structures of the dipeptide complexes **4** and **40** (Fig. 16). The bidentate binding mode via terminal amine and peptide oxygen, as established for the hydroxylamide complex **39** in the solid state, has not yet been found in solution.

The position of the signal for the vanadate–peptide complex in Fig. 14 is not sensitive to pH changes, suggesting, at first sight, that the complex is not susceptible to protonation/deprotonation. Inclusion of emf studies, however, discloses the presence of two complexes, an anionic one (**41a** in Fig. 16) and a protonated, neutral complex (**41b**) (see Eqs. (8a) and (8b)). Complex formation constants *K* obtained from a quantitative evaluation of the ^{51}V and potentiometric data are contained in Eqs. (8a) and (8b). The complete distribution diagram for the system vanadate/alanyl-hisdidine is depicted in Fig. 15. Further support for the participation in coordination of deprotonated amide comes from kinetic studies of the system vanadate + glycyl-L-tyrosine [76]. Two pathways for the complex formation from $H_2VO_4^-$ and Gly-Tyr(1 −) have been detected, one with and one without acid catalysis. The rate constants are 0.017 M^{-2} s^{-1} and 0.025 M^{-1} s^{-1}, respectively. Rate constants for the formation of vanadium(V) complexes with comparable *ON*-functional ligands without an amide amount to ca. 10^4 M^{-1} s^{-1}. Hence, the

involvement of the deprotonation of the peptide-NH as the rate-determining step slows down the reaction by several orders of magnitude.

$$H_2VO_4^- + (\text{Ala-His})^0 \rightleftarrows [\text{complex } \mathbf{41a}]^{-1};\ K = 355\ M^{-1} \quad (8a)$$

$$H_2VO_4^- + (\text{Ala-His})^0 + H^+ \rightleftarrows [\text{complex } \mathbf{41b}]^0;\ K = 3.5 \times 10^9\ M^{-2} \quad (8b)$$

By a comparable methodology, the vanadate–adenosine system (including 5′-derivatives of adenosine [78]) has been evaluated, revealing the formation of a 2:2 complex, where dioxovanadium (VO_2^+) is bonded to the two deprotonated sugar alcohol groups in the 2′ and 3′ positions of ribose [79]. A recent X-ray structure analysis has established a trigonal-bipyramidal geometry for this type of complexes (see **42** in Fig. 16) which, for this reason, is considered an analogue for the transition state of the hydrolytic cleavage of the phospho–ester bond in, e.g. RNA. A transient situation between a trigonal-bipyramidal and trigonal-pyramidal arrangement of the ligand sphere is realised in the dimeric vanadate esters $\{VO(OR)_3\}_2$ (R = CH_2CH_2Cl [82] and *cyclo*-C_5H_9 [83]), where one of the V-(μ-OR) bonds is relatively weak (2.3 Å) (see **43** in Fig. 16). In solution, these dimers are in equilibrium with monomers.

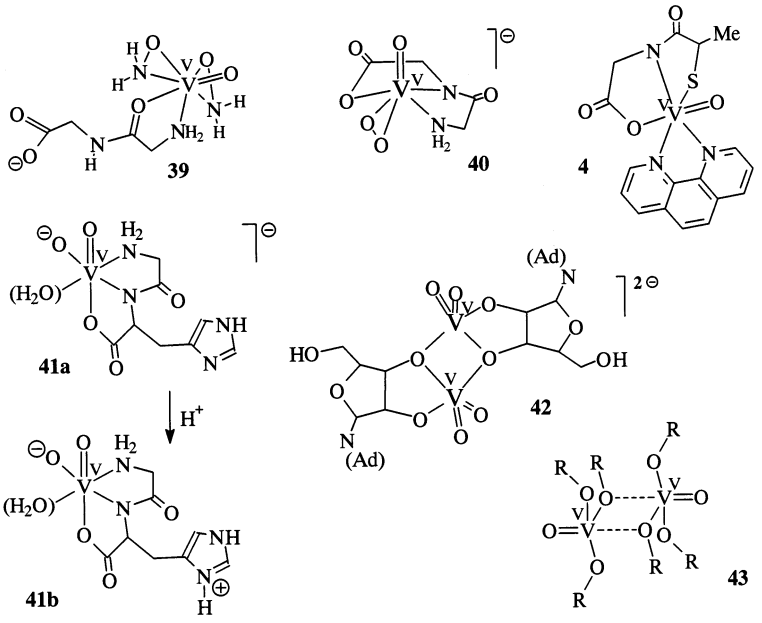

Fig. 16. Complexes formed between vanadate and dipeptides, and vanadate and nucleosides. **39** [62], **40** [80], **4** [19], **41** [72], **42** [81], **43** (R = cyclopentyl) [83]. The structures of **41a,b** have been proposed on the basis of combined emf and multinuclear NMR studies. For the protonation of N^{δ} in histidine see also compound **33** in Fig. 11.

7. Vanadium complexes with non-innocent ligands

Ligands which induce strong charge transfer to a metal centre are sometimes termed non-innocent. In the case of high-valent vanadium, catecholate and hydroximate belong to this ligand category. The relevance of catecholatovanadium complexes for the biological significance of vanadium arises from the possible role of tunichromes in reduction, stabilisation and accumulation of vanadium by tunicates. Several of these tunicates, belonging to the genus *Ascidia*, take up $H_2VO_4^-$ from sea water and accumulate vanadium (up to a concentration of 0.5 M), mainly in the form of V^{III}, in specialised blood cells, the vanadocytes [11,12]. The tunic, and also one particular type of vanadocytes, contains tunichrome, a pigment that consists of subunits related to or constituting dopa (*o*-hydroxytyrosine), linked together by peptide bonds. *o*-Quinones (catecholates) have been shown to be powerful ligands for vanadium ions; an example is the enterobactin complex $[V^{IV}(ent)_3]^{2-}$ [84]. Enterobactin, a siderophore excreted by bacteria to mobilise Fe^{III} from extracellular depots of $Fe(OH)_3$, is a tris(catecholate). Another siderophore, desferrioxamine, has been shown to form a stable complex with vanadium [85]. Desferrioxamines contain hydroximate functions, relating to the bio-relevance of hydroximatovanadium complexes.

^{51}V-NMR spectroscopy is an excellent tool to sort out LMCT complexes of V^V, because these complexes exhibit very unusual ^{51}V shielding conditions [86,87]. While normal penta- to hepta-coordinated vanadium complexes with N_xO_y donor sets give rise to chemical shifts $\delta(^{51}V)$ between ca. -450 and -600 ppm (relative $VOCl_3$) [88], strong deshielding is observed in some hydroxamate (**44** in Fig. 17) and—even more dramatic—catecholate complexes. In the latter case, this is apparently due to participation of a semiquinone form in the resonance hybrid as shown for complex **45a** in Fig. 17. The charge transfer finally leads to the V^{IV} catecholate **45b**, suggesting that an intramolecular redox process is responsible for the reduction of vanadate(V) when incorporated by the ascidian. The $\delta(^{51}V)$ values for **44** (180 ppm) and **45a** (591 ppm) clearly indicate the unusual electronic conditions pertinent to these complexes. At low temperature, three signals arise for **45a** (Fig. 17), i.e. there are at least three structural isomers present in solution [89].

8. Historical perspective and concluding remarks

When vanadium was discovered for the first time—in the year 1803 by the Spanish mineralogist Del Rio in brown lead ore from Mexico [90]—it was given the name erythronium and, shortly thereafter, panchromium as a reference to the beautiful colour changes when it passes through its various oxidation states. However, Del Rio soon was wary of his findings (which he now ascribed the element chromium, which was discovered in 1797) and withdrew his discovery. Almost 30 years later, in the year 1831, vanadium was rediscovered by the Swedish chemist Sefström in remnants of iron ore quarried at the Taberg in Småland. He was a hairsbreadth ahead of Wöhler, who was about to reinvestigate the Mexican

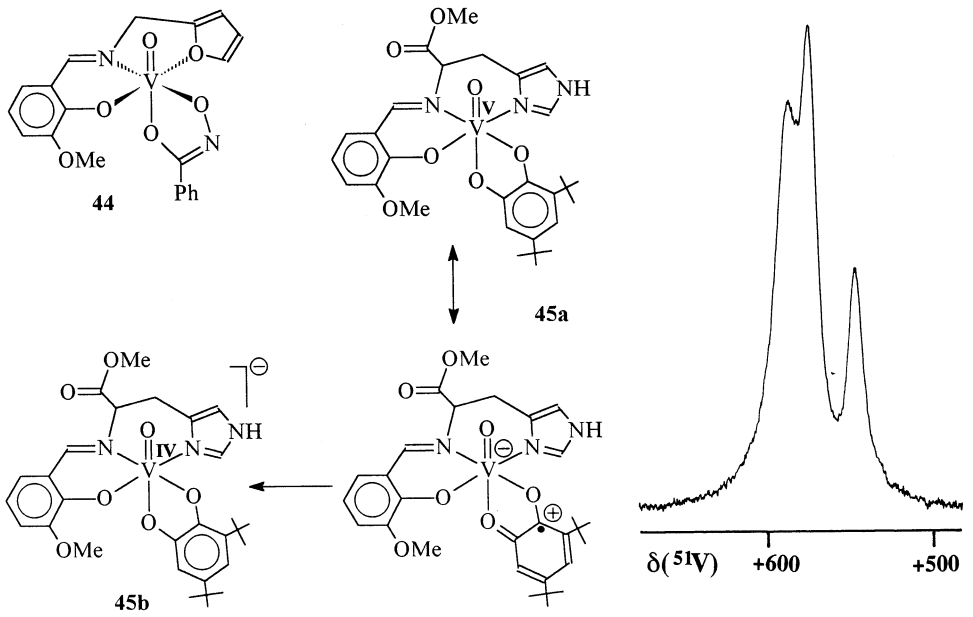

Fig. 17. Vanadium complexes containing the non-innocent ligands benzhydoximate (**44**) and bis(*t*-butyl)-catecholate (**45**). The ^{51}V-NMR spectrum of **45a** (in THF at 220 K) shows the presence of three diastereomers in solution). Taken from Ref. [89].

lead ore with respect to its contents of a new metal [91]. Sefström named the new element vanadin, after the goodess Vanadis, a common by-name for Freija to the Northern Germanic tribes, referring to her beauty.

The importance of vanadium for living organisms was first demonstrated by Henze, who investigated the vanadium contents in the blood of sea squirts [92]. At that time, vanadium was thought to act as an oxygen carrier for the sea squirts and named hämovanadin for this reason. The actual function of vanadium, and the nature of the vanadium compounds present in the vanadocytes remains unsolved to this date. At the beginning of the thirties, Bortels noted that nitrogen fixation by *Azotobacter* is efficient only in the presence of molybdate or vanadate [93]. But it took half a century, until vanadium–nitrogenase was shown to be encoded in nitrogen-fixing bacteria along with the more common molybdenum analogue [94]. A decade earlier, in 1977, the inhibitory effect of vanadate towards phosphatases was established [95]. The second group of vanadium enzymes known to this date, the haloperoxidases, were isolated by Vilter from the sea weed knobbed wrack (*A. nodosum*) in 1983/4 [96]. This was about the time (on occasion of the 2nd International Conference on Bioinorganic Chemistry at the Algarve in 1985), where my group came in touch with the biological aspects of vanadium. Since almost nobody was ready to believe that the new peroxidases contained vanadium(V) in the active site, we inspected the ^{51}V-NMR spectroscopic properties. The ^{51}V-NMR spectra of the bromoperoxidase from *A. nodosum* isolated by Hans Vilter revealed

unusual spectral parameters [97]. Although these results have so far not been confirmed, they initiated intensive investigations into the coordination chemistry of vanadium directed to model the structural and functional peculiarities of its biogenic compounds, set down in several review articles [98]. Many other groups are in this business now, and the overall work on the biological significance of vanadium, which has been retaining its beauty during the decades, has recently been documented in several books [99].

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