

Metallomics

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The role of vanadium in biology

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((Abstract))

Vanadium is special in at least two respects: On the one hand, the tetrahedral anion vanadate(V) is similar to the phosphate anion; vanadate can thus interact with various physiological substrates that are otherwise functionalized by phosphate. On the other hand, the transition metal vanadium can easily expand its sphere beyond tetrahedral coordination, and switch between the oxidation states +V, +IV and +III in a physiological environment. The similarity between vanadate and phosphate may account for the antidiabetic potential of vanadium compounds with carrier ligands such as maltolate and picolinate, and also for vanadium's mediation in cardiovascular and neuronal defects. Other potential medicinal applications of more complex vanadium coordination compounds, for example in the treatment of parasitic tropical diseases, may also root in the specific properties of the ligand sphere. The ease in the change of the oxidation state of vanadium is employed by prokarya (bacteria and cyanobacteria) as well as by eukarya (algae and fungi) in respiratory and enzymatic functions. Macroalgae (sea weeds), fungi, lichens and *Streptomyces* bacteria have available haloperoxidases, and hence enzymes that enable the 2-electron oxidation of halide X^- with peroxide, catalyzed by a Lewis-acidic V^V center. The X^+ species thus formed can be employed to oxidatively halogenate organic substrates, a fact with implications also for chemical processes in the atmosphere. Vanadium-dependent nitrogenases in bacteria (*Azotobacter*) and cyanobacteria (*Anabaena*) convert $N_2 + H^+$ to $NH_4^+ + H_2$, but are also receptive for alternative substrates such as CO and C_2H_2 . Among the enigmas to be solved with respect to the utilization of vanadium in nature is the accumulation of V^{III} by some sea squirts and fan worms, as well as the purport of the nonoxido V^{IV} compound amavadin in the fly agaric.

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1. General and background

Earth's crust, plus the water reservoirs and the atmosphere, contain an average of 135 ppm vanadium (exceeding vanadium's concentration in the Universe by a factor of ca. 135). This makes vanadium the 21st most abundant elements in the outer regions of our planet.

Generally, vanadium is rather dissipated, i.e. vanadium-based minerals are comparatively rare. A "famous" representative is vanadinite, a lead *orthovanadate* of formula $\text{PbCl}_2 \cdot 3\text{Pb}_3(\text{VO}_4)_2$ that is closely linked to vanadium's first discovery by Andrés Manuel del Río y Fernandez in Mexico in 1801.¹ Enrichment of vanadium has been observed in soils and rocks in volcanic areas, and in crude oil, oil-shales, asphalts, peat, and bitumen. In crude oil, vanadium contents – with vanadium present in the form of VO^{2+} -porphyrins – can go up to 0.12%. This accumulation of vanadium goes back to the extraction of VO^{2+} ("vanadyl") from shale that is being pervaded by kerosene. In coal bottom ash, total vanadium concentrations can go up to 0.7 g per kg dry weight.² The occurrences of "fossil" vanadium pose potential environmental and health problems, in as far as burning coal and oil produces vanadium oxides that become absorbed to dust particles. As detailed below, vanadium oxides can cause health hazards; furthermore, vanadium oxides are powerful catalysts in the oxidation of, for example, SO_2 to SO_3 (and hence sulfuric acid).

Sea water contains vanadium in the form of ion pairs $\text{Na}^+\text{H}_2\text{VO}_4^-$ at a concentration typically between 30 to 35 nM, making vanadium the second-to-most abundant transition metal in the oceans, overtopped only by molybdenum in the form of molybdate MoO_4^{2-} at concentrations of 100 nM. This comparatively high abundance of vanadate(V) in sea water does have consequences with respect to vanadium's utilization by macro-algae, and thus indirectly also for a role of vanadium in the global ozone balance – as will be detailed later in this review. Other marine organisms, namely sea squirts (ascidians) and some fan worms, also make recourse to vanadate, although without any apparent biological benefit.

The average vanadium concentration in fresh water, ground water and potable water is 10 nM, with peak concentrations in volcanic areas going up to 2.5 μM . The average vanadium content in edibles, where vanadium is mainly present in the form of vanadyl species, amounts to 5-30 $\mu\text{g kg}^{-1}$. At ambient conditions, the daily oral intake of vanadium via potable water and food varies between 10 μg and 2 mg. This is clearly beyond the no-effect level of 10 mg vanadium per day and per kg body mass.

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After oral uptake of vanadium compounds, speciation occurs by the saliva, in the stomach and the intestinal tract. The main part of vanadium is thus converted to sparingly soluble $\text{VO}(\text{OH})_2$, most of which is excreted via the faeces, minimizing or even excluding adverse effects that otherwise might be caused by unphysiologically high vanadium levels. Vanadate(V) is more easily resorbed than soluble vanadyl species, and this can principally cause health problems, for example in households with lead water pipe systems, where drinking water enriched with phosphate can contain appreciable amounts of vanadate: phosphate remobilizes vanadate from otherwise insoluble lead vanadate deposited in the wall of the pipe system.³ As noted above, an additional source for vanadium intake are aerosols in the breathing air. In urban areas, vanadium contents in the breathing air can go up to 10^3 ng m^{-3} of vanadium, and hence two to three orders of magnitude more than in rural areas. Incomplete combustion of fossil fuels is a major source for vanadium oxides absorbed to particulate matter in the air. In the alveoli of the lung, vanadium oxides VO_x can be converted (oxidatively) to vanadate and thus become resorbed. In addition, *direct* pulmonary problems can result from high aerial loads of VO_x ,^{4a} a main issue for labourers exposed to excessive aerial VO_x concentrations at the working place. The maximum allowable concentration (MAC) of V_2O_5 at the workplace has been set to 0.05 mg m^{-3} . For a recent review of the direct and indirect toxicity of V_2O_5 see Fortoul *et al.*^{4b}

Once in the blood stream, vanadium – in the form of vanadate and vanadyl – binds to serum proteins, in particular to transferrin,^{5a} an issue that originally was attended to by Chasteen.^{5b, c} Vanadium is then distributed to the tissues of the inner compartment (heart, liver, and kidney) and the outer compartment (brain, muscle, adipose tissues). All in all, vanadium contents in the blood are reduced to about 30% within one day.⁶ Bone, however, and to some extent also the kidneys, can hold back vanadate/vanadyl. In the apatite of the bones, vanadate can substitute for phosphate; here, the half-life of vanadium amounts to about 5 days.⁷ Elimination of resorbed vanadium occurs via the urinary tract; in the kidneys, VO^{2+} can be retained intermittently, for example by coordination to dangling NH_2 groups of proteins.

The ability of vanadate to substitute for phosphate in apatite points to a pronounced similarity between vanadate and phosphate (Fig. 1, **a**), and hence towards a possible *general* role of vanadate in physiological processes involving phosphate. The tetrahedral anions vanadate and phosphate are in fact structural analogs of almost the same size, with a volume of the circumscribing spheres of 125 and 102 \AA^3 , respectively.⁸ Vanadate can thus easily substitute for phosphate in enzymes such as phosphatases and kinases. But this fact also

points towards distinct *differences* between vanadate and phosphate: vanadium in vanadate easily attains the stable coordination number five – commonly in a somewhat distorted trigonal-bipyramidal coordination environment (Fig. 1, **b**) – while penta-coordination is just a transition state in the case of phosphate. Consequently, the replacement of phosphate for vanadate results in the inhibition of the enzyme, as initially documented by Chasteen^{5b} and established for the inhibition of a Na,K-ATPase by Cantley almost four decades ago.⁹ With an association constant of $2.4 \times 10^8 \text{ M}^{-1}$, vanadate forms a stable five-coordinate complex with the ATPase, involving bidentate binding of an aspartate carboxylate. An additional feature that distinguishes phosphate and vanadate at physiologically relevant concentrations is the protonation state at about neutral pH: at pH 7, vanadate is almost exclusively present in its diprotonated form, eqn (1a), while phosphate exists as a mix of mono- and dihydrogenphosphate, eqn (1b). Further, in contrast to phosphate, vanadate(V) is susceptible to reduction to oxidovanadium(IV), eqn (2), and vanadium (III).

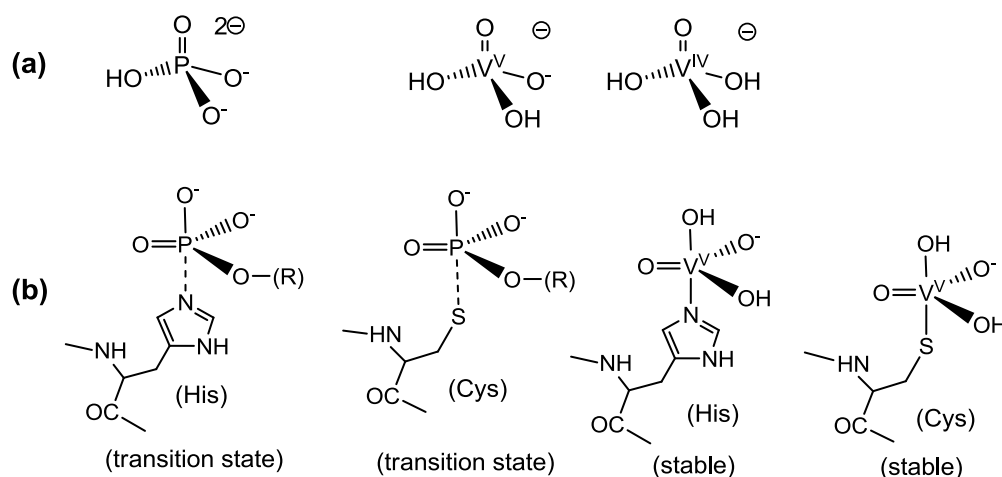
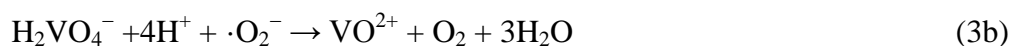


Fig. 1 Analogies and differences between phosphate and vanadates. **(a)** The predominant protonation states of phosphate and of the vanadates(V) and -(IV) at neutral pH. Due to the low solubility of $\text{VO}(\text{OH})_2$, dissolved vanadate(IV) H_3VO_4^- (the coordination sphere of V^{IV} can be expanded by aqua ligands) is restricted to the nanomolar concentration range. **(b)** Penta-coordinate species evolving from the interaction between phosphate (and phosphate esters) or vanadate with peptide/protein residues: phosphate forms labile transition states only, symbolized here by a dashed P---N bond to histidine or a P---S bond to cysteine (serinate – not shown – is a third alternative), while vanadate ascertains stable complexes. Examples are

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4 the binding of vanadate to a histidine residue in vanadate-dependent haloperoxidases (section
5 4.2) and in rat prostate acid phosphatase,^{10a} and the coordination of vanadate to a cysteinate
6 residue in phosphotyrosyl phosphatase.^{10b} This coordination mode has also been invoked for
7 the inhibitory effect of vanadate towards intracellular protein tyrosine phosphatase in the
8 context of the insulin enhancing properties of vanadate (section 5).¹¹ The hydroxide in the
9 apical position can be replaced by, for example, tyrosinate. For an overview of structural
10 details, see Crans *et al.*¹²

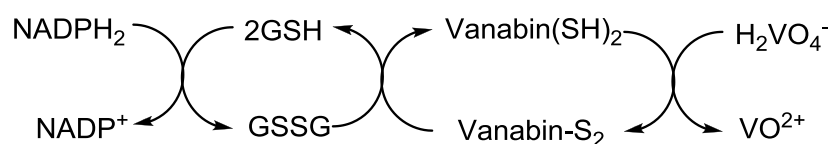
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16 The most common oxidation states of vanadium in biological systems are the +V (d⁰)
17 and +IV (d¹) states, but the +III (d²) is also principally available and has been shown to be
18 realized in the final storage form of vanadium in ascidians (section 2). In vanadium-dependent
19 nitrogenase (section 4.1), the +II state supposedly also comes in; this oxidation state of
20 vanadium is otherwise unstable at physiological and environmental conditions. In close
21 relationship to the ease of redox interconversion between vanadium(V) and –(IV), vanadium
22 can play a crucial role in balancing the level of reactive oxygen species. As examples, the
23 generation of the hydroxyl radical as initiated by VO²⁺, and the elimination of the peroxy
24 radical by vanadate, are depicted in eqn (3).
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35 36 37 38 39 **2. Unspecific accumulation of vanadium compounds in living organisms**

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42 Three groups of organisms have so far been identified that accumulate vanadium without any
43 apparent benefit, viz. (i) several *Amanita* mushrooms such as the fly agaric, (ii) marine
44 polychaeta fan worms, and (iii) ascidians. In ascidians, specialized blood cells termed
45 vanadocytes take up vanadium; the highest vanadium contents have been found in *Ascidia*
46 *gemmate*, with vanadium concentrations up to 350 mM – hence enrichment from sea water by
47 a factor of 10⁷! The role of vanadium is obscure; since vanadium is toxic at higher
48 concentrations, the accumulation in ascidians (and some fan worms) might thus hint towards a
49 function as poison for potential predators. In any case, vanadium does not take over a role in
50 oxygen transfer, as originally suggested, and coined by the term hemovanadin, by Henze, who
51 was the first to provide evidence for the accumulation of vanadium in the blood of ascidians
52 more than a century ago,^{13a} an area that was later pioneered by Kustin^{13b, c} and, more recently,
53 by Michibata *et al.* (*vide infra*).
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Vanadium enters the ascidians, assisted by Na^+ -dependent phosphate transporters, as vanadate(V) $\text{H}_n\text{VO}_4^{(3-n)-}$ ($n = 2, 1$), which is then reduced to V(IV) (VO^{2+}) and further to V(III), the storage form in the vanadocytes, essentially $[\text{V}(\text{H}_2\text{O})_5\text{HSO}_4]^{2+}/[\text{V}(\text{H}_2\text{O})_6]^{3+}$. Several key proteins are related to the accumulation and reduction of vanadium, among these so-called vanabins. Vanabins are low molecular mass proteins rich in cysteinyl residues. A well investigated representative is vanabin2, isolated from *Ascidia sydneiensis samea*. Vanabin 2 is a 13.2 kDa protein consisting of 120 amino acids,¹⁴ 18 of which are cysteines that form nine disulfide bonds. Vanabin2 acts as a reductase for vanadate (Scheme 1) and intermittent store for vanadyl VO^{2+} . The reductase activity has been traced back to cysteine residues,¹⁵ the binding of VO^{2+} is associated with dangling amino groups provided by lysine and arginine residues. The final reduction step ($\text{VO}^{2+} \rightarrow \text{V}^{3+}$) so far remains obscure.



Scheme 1 The reduction of vanadate(V) as catalyzed by vanabin2: In a first step, the reduced form of nicotine-adenine dinucleotide, NADPH_2 , reduces glutathione disulfide GSSG to glutathione GSH. In a second step, the disulfide form of vanabin2 is reduced to the thiol form which, in the final step, reduces vanadate to vanadyl VO^{2+} . Based on ref. 15.

Several representatives of the genus *Amanita* contain a non-oxido vanadium(IV) complex $\text{Ca}(\text{H}_2\text{O})_5[\Delta\text{-V}(\text{S,S-hidpa})_2]$ termed amavadin (Fig. 2), in which vanadium is octa-coordinated to two hidpa^{3-} ligands, where the hidpa^{3-} is short for *N*-hydroxyimino-2,2'-diisopropionate(3-). The bulb and the lamella of the mushroom are particularly rich in vanadium; in *A. muscaria*, the fly agaric (also known as toad stool), vanadium levels can go up to 1 g amavadin per kg of dry mass, hence an enrichment by a factor of 10^3 to 10^4 with respect to the average vanadium concentrations in soil. The biological function of amavadin is elusive; it has been proposed that amavadin is an evolutionary overcome relic of an oxidase or peroxidase. The amavadin anion in its oxidized (vanadium(V)) form, $[\Delta\text{-V}(\text{S,S-hidpa})_2]^-$, can in fact act as a peroxidase and, in the absence of a substrate, as a catalase.¹⁶ The catalase activity is depicted in eqn (4), the peroxidase activity in the presence of thiols, resulting in the formation of disulfides, in eqn (5). In eqns (4) and (5), the reduced and oxidized forms of amavadin are symbolized by $\{\text{V}^{\text{IV}}\}$ and $\{\text{V}^{\text{V}}\}$. Mechanistically, the one-electron oxidation of thiols, such as mercapto-methylacetate, likely proceeds via a protonated V^{V} and a V^{IV} -thiol

intermediate¹⁷ as exemplified in Fig. 2. Interestingly, the protonated V^V form of amavadin, H⁺[V(hidpa)₂]⁻, also exerts bromoperoxidase activity,¹⁸ an ability otherwise restricted to naturally occurring vanadate-dependent bromoperoxidases; section 4.2.

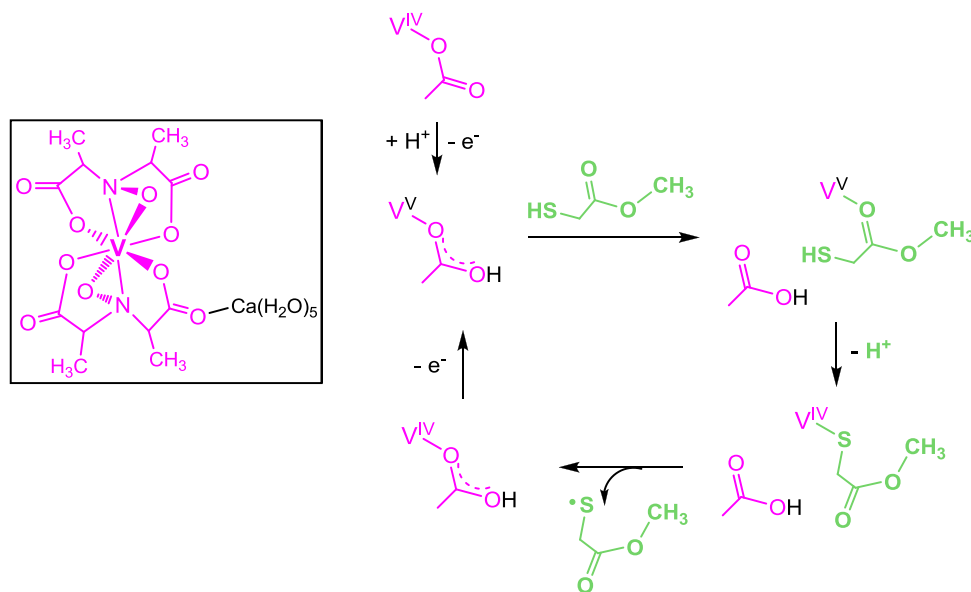
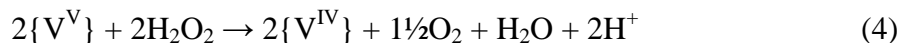


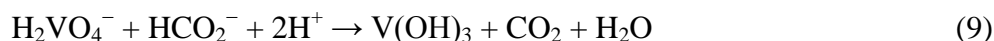
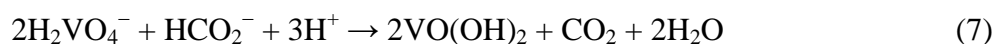
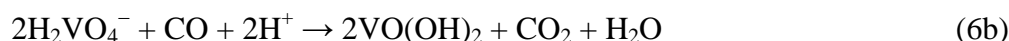
Fig. 2 The vanadium compound amavadin (framed) from the fly agaric (*Amanita muscaria*), and intermediate steps in the oxidation of mercapto-methylacetate (green), catalyzed by amavadin (purple) as suggested by DFT calculations (from ref.¹⁷; modified).

3. Vanadium in bacterial respiration

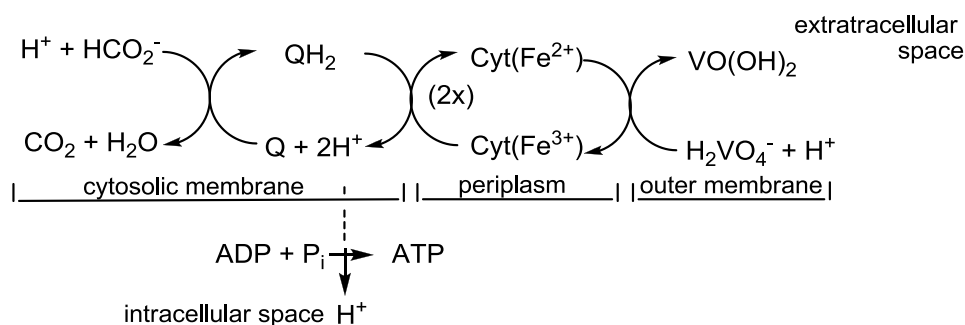
A whole array of bacteria can employ vanadium in varying biological functions.^{19a} Well documented examples are the bacterial vanadium-dependent nitrogenases and haloperoxidases (both enzymes will be dealt with in detail in section 4), and bacteria that resort to vanadate(V) as an electron acceptor in respiration. Vanadium has also been reported to optionally replace molybdenum in the periplasmatic nitrate reductase of *Pseudomonas isachenkovii*:^{19b} nitrate reductases catalyze the two-electron reduction of nitrate to nitrite.

The facultative anaerobic chemolithotrophic bacterium *P. isachenkovii* reduces vanadate, primarily to VO²⁺ in the form of sparingly soluble VO(OH)₂, resorting to, inter alia, H₂ or CO as electron donors in vanadate reduction, eqns (6a) and (6b).²⁰ Other bacterial strains can equally use vanadate as an electron acceptor in respiratory and/or dissimilatory

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3 reduction. *Respiratory* reduction refers to a process coupled to proton translocation and thus
4 ATP generation and growth, while *dissimilatory* reduction occurs without a proton motive
5 force. Specific strains of *Shewanella oneidensis*, a Gram-negative soil bacterium, utilizes
6 lactate and formate as electron sources in respiratory reduction,²¹ eqn (7), while *Vibrio*
7 *parahaemolyticus*, thriving in brackish salt water and causing gastrointestinal dysfunctions
8 when ingested, promotes dissimilatory vanadate reduction by making recourse to glycerol or
9 formate.²² The strict anaerobe *Geobacter metallireducens* effectively employs metal ions and
10 metalates with the metal in a high oxidation state (including vanadate,²³ eqn. (8)) as electron
11 acceptors in the oxidation of organics such as fatty acids and alcohols, and thus contributes to
12 the detoxification of ground water with high metal ion loads. Finally, *P. isachenkovii*, as well
13 as vanadate-respiring anaerobic bacteria isolated from deep sea hydrothermal vents,²⁴ have
14 been shown to partially reduce vanadate(V) to vanadium(III), eqn. (9).
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37 In the case of *S. oneidensis*, a terminal vanadate reductase associated with the outer
38 membrane of the organism catalyzes the reduction of vanadate to vanadyl. VO(OH)₂ becomes
39 deposited mainly in the periplasm and at the outer membrane. The electrons for the reduction
40 of H₂VO₄⁻ to VO²⁺ are commonly delivered through the oxidation – in the cytosolic
41 membrane – of lactate to pyruvate, or of formate to CO₂. The electrons are then shuttled to the
42 outer membrane by cytochrome-c type haem proteins across the periplasmic space, and
43 finally to membrane-associated vanadate. Scheme 2 provides a simplified picture of this
44 situation.
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Scheme 2 A simplified view of the electron transfer across the cellular membrane, starting with the oxidation of formate (inner membrane), and terminating with the reduction of vanadate at the outer membrane. Q/QH₂ is menaquinone/-hydroquinone. The electron transport from the inner (the cytosolic) to the outer membrane is accomplished via a cascade of cytochrome-*c* type haem proteins. Vanadium ends up in a mineralized form based on V^{IV}O(OH)₂. The H⁺ transport into the intracellular space is coupled to the formation of energy-rich adenosine triphosphate (ATP) from ADP and inorganic phosphate HPO₄²⁻ (P_i).

In addition to bacteria, mesophilic and thermophilic methanogenic archaea such as *Methanothermobacter thermautotrophicus* can also reduce V^V to V^{IV}, diverting the electron transfer in such a way that methanogenesis is inhibited.²⁵ The reduction of vanadate by prokarya, resulting in the formation of vanadyl (and, in part, also of V^{III}) has likely contributed – and still contributes – to the mineralization of soluble vanadate. Minerals such as häggite V^{III}O(OH)·V^{IV}O(OH)₂, simplotite CaV^{IV}₄O₉ and sherwoodite Ca₉Al₂V^{IV}₄V^V₂₄O₈₀·xH₂O are examples.

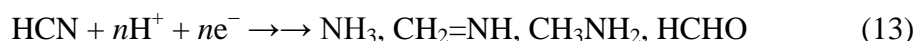
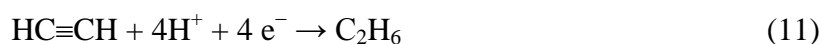
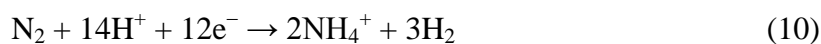
4. Nitrogenases and haloperoxidases

To date, the only indubitably established naturally occurring vanadium-dependent enzymes are either haloperoxidases or nitrogenases. Vanadium *nitrogenases* VNases can be present, along with iron-only nitrogenases and the (phylogenetically younger and far more prevalent) molybdenum nitrogenases, in bacterial strains belonging to the genus *Azotobacter* and in cyanobacteria of the genera *Anabaena* and *Nostoc*. The vanadium-based *Azotobacter* nitrogenase is more effective than its molybdenum analogue at low temperatures, and is predominantly expressed when Mo is limited. Vanadate-dependent *haloperoxidases* VHPOs have been found in marine macroalgae, in terrestrial fungi, lichens, *Streptomyces* bacteria and in cyanobacteria, in part along with heme-dependent peroxidases.²⁶ VHPOs are rather wide-

spread and are directly involved in the utilization of halides in aquatic (essentially marine) environments either for synthesizing halogenated organics or, in the case of chloroperoxidases, either in the organism's defense against bacterial and viral affliction, or to get access to a host. Indirectly, VHPOs are also involved in atmospheric issues, the removal of ozone in particular (section 4.3).

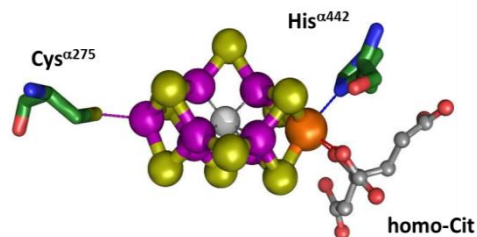
4.1 Vanadium nitrogenases

Proteobacteria such as the soil bacteria *Azotobacter vinelandii* and *A. chroococcum* are equipped with VNase, as are the filamentous cyanobacteria *Anabaena nostoc*,²⁷ *A. azotica*, and *A. variabilis*. More recently, a VNase has also been detected in lichens of the genus *Peltigera*,²⁸ thriving in high latitude ecosystems. These lichens contain cyanobacterial symbionts belonging to the genus *Nostoc* of the family *Nostocaceae*, related to *Anabaena*, another genus that is a member of this specific family of cyanobacteria. VNases catalyze the reduction of N₂ to NH₄⁺, thus bio-mimicking the Haber-Bosch synthesis. In the natural system, the reduction of N₂ to NH₄⁺ (and commonly some hydrazine) is coupled to the reduction of H⁺ to H₂,^{29a} the overall ATP-driven reaction is represented by eqn (10). Several substrates other than N₂ (and H⁺) can also undergo nitrogenase-catalyzed reduction; examples are CO and acetylene,^{29b} eqns (11) and (12), and HCN.^{29c} Acetylene is reduced to ethane, eqn (11), CO to ethene, ethane, propene and propane, eqn. (12). The reduction of CO is reminiscent of the Fischer-Tropsch process. The reductive conversion of HCN, formulated non-stoichiometrically in eqn. (13), affords several products, among these ammonia, methyleneimine, methylamine and formaldehyde.



The VFe-protein and the MoFe proteins are biochemically similar, although they differ somewhat in their substructures, viz. $\alpha_2\beta_2$ for the MoFe protein and $\alpha_2\beta_2\delta_2$ for the VFe-protein. Once isolated from the bacteria, VNases are sufficiently less robust than their molybdenum analogues, and so far direct structural information (i.e. information obtained from X-ray structure analyses) is not available. However, indirect information from various

sources (such as extended X-ray absorption, electron paramagnetic resonance EPR, Mößbauer, and magnetic circular dichroism spectroscopies) indicates a buildup similar to that of the Mo-nitrogenases.³⁰ Correspondingly, the central unit of the vanadium-iron protein (Fig. 3) – the so-called *M* clusters or FeVCo, where the direct reduction of N₂ to NH₄⁺ occurs – is a cage system formed by seven iron ions plus one vanadium ion.



The metal centres are bridged by nine S²⁻. Six of the iron centres of the Fe₇ cage are additionally linked to an interstitial light atom that, in analogy to the Mo-nitrogenase, supposedly is carbon in the form of carbide μ₆-C⁴⁻.³¹ The cluster is connected to the protein matrix via a cysteine (coordinated to one of the iron centres), and the Nε of a histidine residue, coordinated to vanadium. A homocitrate completes the coordination sphere of vanadium. The EPR spectra of the FeVCo adopt a pattern that is characteristic of a spin *S* = 3/2 state for the vanadium centre, hence suggesting high-spin V^{+II}.^{30b}

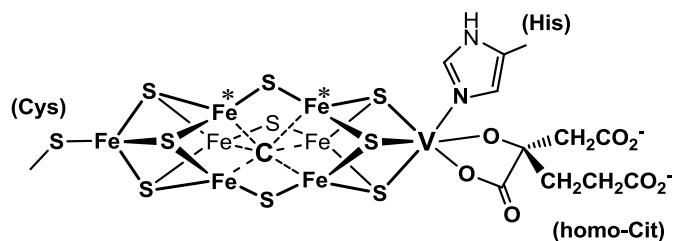


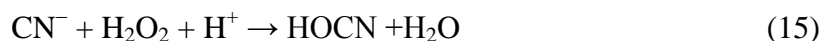
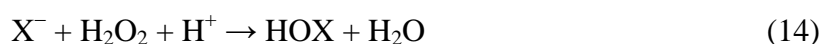
Fig. 3 The structure of the *M* cluster {VFe₇(μ₆-C)(μ₂-S)₃(μ₃-S)₆} of vanadium nitrogenase. Left: schematic view, right: ball-and-stick representation (adapted with permission from ref. 30(b); © Am. Chem. Soc.). Potential sites for (side-on) binding of N₂ are the iron centres labeled with an asterisk.³² Electrons for the N₂ reduction are delivered via an adjacent [4Fe-4S] ferredoxin-type cluster. The complete system is encoded by the *vnfHDK* genes.

4.2 Vanadate-dependent haloperoxidases

Along with co-factor free and a haem-based haloperoxidases, vanadate-dependent haloperoxidases VHPOs represent a third powerful regime for the oxidation, by hydrogen peroxide, of halides. VHPOs are differentiated with respect to their primary halide substrate(s): iodoperoxidases VIPOs oxidize iodide only, bromoperoxidases VBrPOs oxidize iodide and bromide (and also, to some extent, chloride), and chloroperoxidases VCIPOs have a sufficiently high oxidation potential to oxidatively attack chloride along with iodide and bromide. In contrast (and somewhat surprisingly), a vanadate-substituted acid phosphatase

isolated from the embryonic axes of the kidney bean *Phaseolus vulgaris* exhibits chloroperoxidases activity, but no bromo- and iodoperoxidases activity.³³

Common substrates for marine VHPOs are the halides I^- , Br^- and Cl^- ; these ions are present in sea water at average concentrations of 0.47 μM (iodide), 0.82 mM (bromide) and 0.55 M (chloride). Other substrates, the pseudohalides cyanide and thiocyanate in particular, are also oxidized, as are organic compounds such as sulfides R_2S . The latter are oxidized to sulfoxides; in the case of prochiral sulfides (RSR') as substrate, the oxidation occurs enantioselectively. This is noteworthy since the vanadate-based reaction centre itself (vide infra) is non-chiral, suggesting influencing factors that go back to the active site protein pocket. The oxidant is hydrogen peroxide H_2O_2 , the concentration of which in sea water can go up to 0.25 μM , but is otherwise quite variable. All of these reactions are two-electron oxidations. Eqns (14) to (17) exemplify a selection of the oxidation reactions; X^- represents a halide. In reactions (14) to (16), protons are consumed; the oxidation of sulfides, eqn (17), affords just catalytic amounts of H^+ . "HOX" in eqn (14) actually is an equilibrium mixture of HOX, X_2 and X_3^- . In the absence of a substrate, singlet oxygen 1O_2 is released.



The oxidation of halide exemplified by eqn (14) is a generalization in as far as free hypohalous acid HOX (or hypohalite XO^-) does not necessarily emerge. Rather, a substrate can be halogenated directly, presumably via the intermediate formation of an " X^+ " species (such as Br_2 or Br_3^- in the case of $X^- =$ bromide).³⁴ For $X = Cl$, however, hypochlorous acid HOCl is commonly directly employed. VCIPOs are predominantly found in *Streptomyces* bacteria,³⁵ where they serve as chlorinating agents for complex organics, and in terrestrial fungi. Fungi such as *Curvularia inaequalis*³⁶ can use HOCl to oxidatively degrade the lignocellulose in the cell wall of their "host", thus allowing access of the fungal hypha to the host's intracellular space.³⁷ The *C. inaequalis* VCIPO has also been shown to possess antimicrobial activity, for example against the intestinal bacterium *Enterococcus faecalis*.^{38a} Furthermore, an alkophilic mutant of the VCIPO has a broad antimicrobial activity against Gram-negative and Gram-positive bacteria, and also exhibits virucidal activity.^{38b}

In the VHPOs, vanadate H_2VO_4^- is linked to the N ϵ of a histidine in the enzyme's active centre, and is additionally stabilized through various hydrogen bonding interactions to neighbouring amino acid residues, as sketched in Fig. 4 for the VBrPO from the marine green macroalga *Ascophyllum nodosum* (known as knotted wrack or knotted kelp) and the red seaweed *Corallina pilulifera*. The vanadium(V) centre is in a trigonal-bipyramidal coordination environment, with histidine in one of the axial positions. *A. nodosum* contains two homologous bromoperoxidases, VBrPO(*AnI*) and VBrPO(*AnII*), both of which have been thoroughly characterized: VBrPO(*AnI*) is a homodimer of 557 amino acids per subunit,³⁹ and VBrPO(*AnII*) is a hexamer with 641 amino acids in each subunit.⁴⁰ The sequence homology for the two homologues is 41 %. The coordination environment of vanadium in the VCIPOs⁴¹ is identical to that of the VBrPOs. Interestingly, the buildup of the active centre in VHPOs is very much the same as in rat prostatic acid phosphatase,¹⁰ when phosphate is replaced by vanadate (Fig. 4, right).

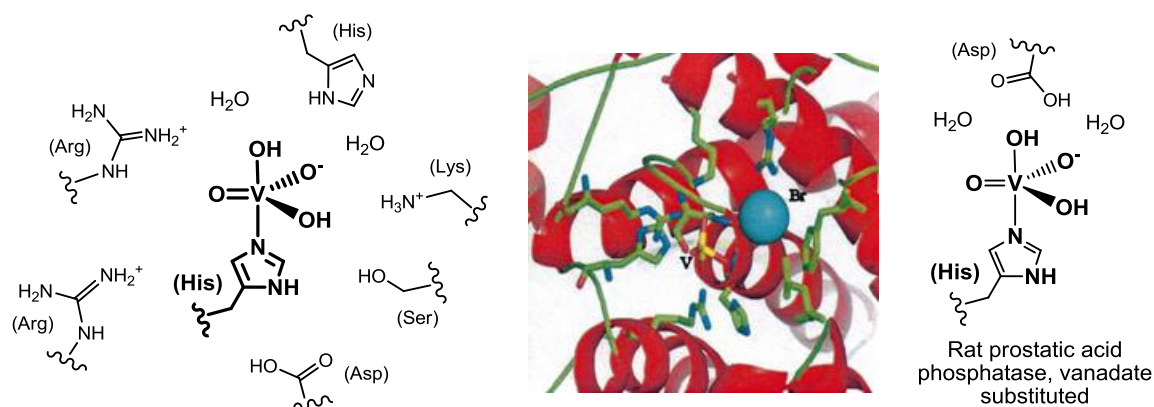
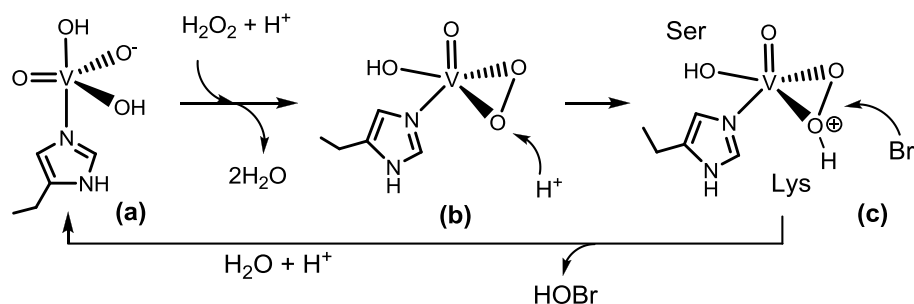


Fig. 4 Left: The vanadate(V) center of the vanadate-dependent bromoperoxidase from *A. nodosum*. Most of the close-by amino acid residues in (hydrogen-bonding) contact with the active center, plus two water molecules, are shown. Centre: The environment of vanadate (yellow), and the location of bromide (blue) in the *C. pilulifera* peroxidase.⁴² Bromide is positioned within hydrogen bonding distance (ca. 3.0 Å) between vanadate and an arginine residue. Reproduced with permission, © Elsevier. Vanadate, deeply buried in the protein, is accessible via a positively charged channel. Right: The vanadate center in the vanadate variant of rat prostatic acid phosphatase.

The substrate halide does not bind directly to vanadium. Rather, it interacts peripherally with the active centre, as shown in Fig. 4. During turnover, peroxido and hydroperoxido intermediates are involved; here, vanadium is in an environment in-between a trigonal-bipyramidal and tetragonal-pyramidal arrangement,⁴³ Scheme 3. The hydroperoxido

intermediate is attacked by the halide, generating hypohalous acid and thus restoring the starting situation; for the net reaction see eqn (14). The oxidation state of vanadium (+V) does not change during turn over; the catalytic V^V centre thus functions as a Lewis acid rather than a redox catalyst.



Scheme 3 Proposed mechanism for the 2-electron oxidation of bromide to a Br^+ species such as hypobromous acid $HOBr$.^{43a} Serine⁴⁴ and lysine (and/or arginine) play an intrinsic role in activating the (hydro)peroxido intermediate and as a director for bromide.

To speak in a general way, oxidovanadium(V) compounds are inherently active in the catalysis of oxidation reactions. The peroxidase activity of the VHPOs in particular has thus inspired various groups to “copy” the naturally occurring enzyme by devising molecular models in which vanadium is in a similar arrangement as in the VHPOs. Even simpler vanadium compounds, such as nano-particulate vanadium pentoxide $n-V_2O_5$ has equally been shown to be an efficient oxidant: $n-V_2O_5$ counter-acts biofouling, for example of submerged ships’ hulls.⁴⁵ Molecular “models” for the active centre of VHPOs can thus greatly deviate from the actual vanadium environment in VHPOs. A selection of models is collated in Fig. 5. The oxidovanadium(V) complex **1** in Fig. 5 is a comparatively close model of the active centre of the peroxidases in as far as vanadium is in an approximately trigonal bipyramidal environment constituted of one nitrogen donor opposite of the oxido ligand, and three oxygen donors in the equatorial plane. The complex catalyzes the enantio-selective oxidation of prochiral sulfides to chiral sulfoxides.⁴⁶ Complex **2** is another example where an NO_4 coordination sphere is realized; **2** catalyzes peroxidative brominations.⁴⁷ The complexes **3**⁴⁸ and **4**⁴⁹ exemplify crystallographically characterized peroxido intermediates.

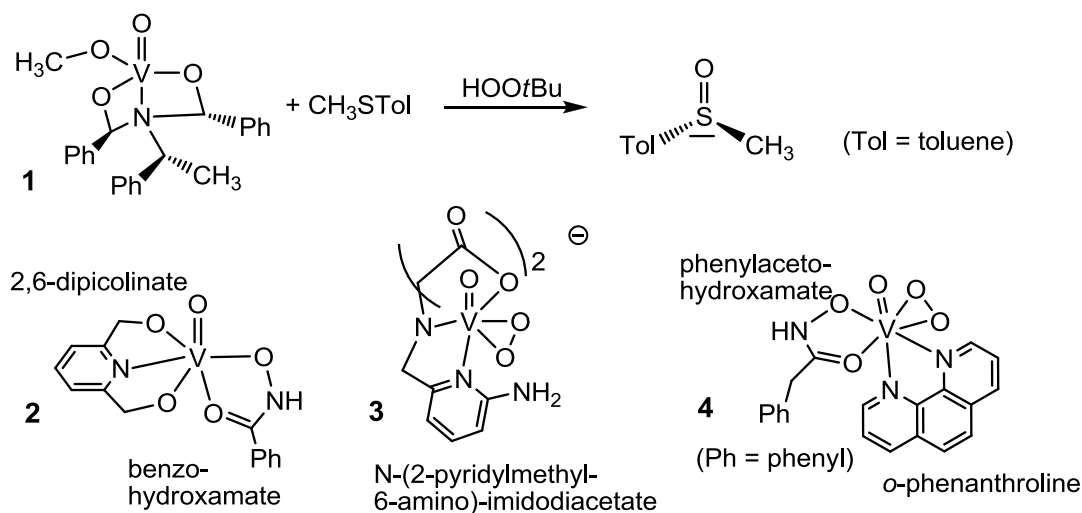
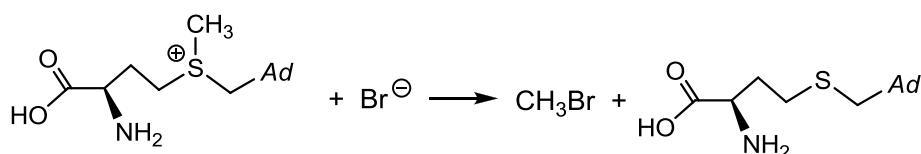


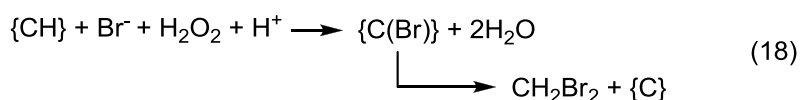
Fig. 5 A selection of vanadium complexes that model the active center of vanadate-dependent haloperoxidases (**1** and **2**), and the intermediate peroxido state (**3** and **4**).

4.3 Impact on atmospheric chemistry

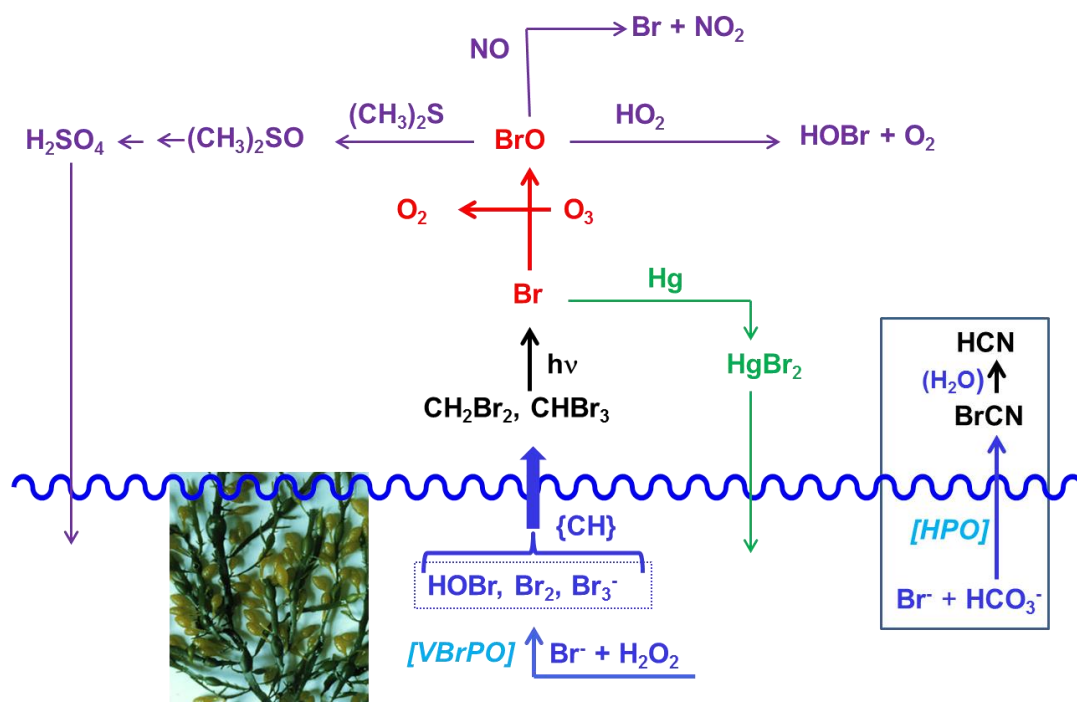
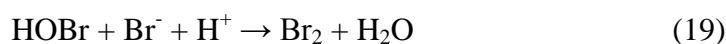
Brown macroalgae such as *A. nodosum* and *Laminaria digitata*, as well as the red seaweed *Delisia pulchra* efficiently brominate a broad variety of organic substrates; these products of halogenation include halomethanes. Monosubstituted halomethanes are preferentially generated in the frame of a nucleophilic attack of halide to the CH_3S^+ site of S-adenosylmethionine, Scheme 4,⁵⁰ and hence without participation of VHPOs, while the synthesis of *di*- and *tri*-substituted halomethanes (including hetero-substituted ones such as CHBr_2I and CHBr_2Cl) is catalyzed by VHPOs with recourse to reactive dissolved organic matter $\{\text{CH}\}$ ⁵¹ and (hydrogen)peroxide. $\{\text{CH}\}$ is available externally from environmental sources or produced as a by-product in photosynthesis. For a net reaction course yielding dibromomethane see eqn (18).⁵² It should be noted that, along with the marine production of halogenated methanes, terrestrial contributions are involved, in particular soils in tropical areas.



Scheme 4 The formation of monobromomethane from bromide and S-adenosylmethionine; *Ad* = adenosine.



In Fig. 6, several of the atmospheric processes involving halomethanes are exemplified for the bromomethanes $\text{CH}_{4-x}\text{Br}_x$ ($x = 2, 3$) as primary products introduced into the troposphere; for similar scenarios with iodomethanes $\text{CH}_{4-x}\text{I}_x$ see for example ref.⁵³. The overall coastal flux of $\text{CH}_{4-x}\text{Br}_x$ has been estimated to $2.5 \cdot 10^9$ moles per year.^{54a} Accordingly, light-induced photolysis in the troposphere generates Br radicals that catalyze the depletion of ozone (red in Fig. 6), forming, along with O_2 , bromine monoxide BrO. About 20-30% of the atmospheric ozone depletion is attributed to BrO. Br is then partially regenerated from BrO via $2\text{BrO} \rightarrow 2\text{Br} + \text{O}_2$ or $\text{BrO} + \text{O}_3 \rightarrow \text{Br} + 2\text{O}_2$ (not shown in Fig. 6). BrO can then react with atmospheric constituents such as hydroperoxide radicals HO_2 , dimethyl sulfide, and nitrous oxide.^{54a} These reaction paths are sketched in mauve in Fig. 6. The hypobromous acid recovered in the reaction between BrO and HO_2 can additionally be involved in a symproportionation with bromide and thus recover bromine Br_2 , eqn (19). Photolytic splitting by light then regenerates bromine radicals. This reaction sequence is occasionally referred to as “bromine explosion”.^{54b}



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4 **Fig. 6** Selected atmospheric products/processes, exemplified here for bromomethanes, the
5 formation of which is initiated by apoplastical bromoperoxidase VBrPO such as present in the
6 brown macroalga *A. nodosum* (shown). For the release of the comparable iodomethanes by
7 the brown alga *Laminaria digitata* see ref.⁵⁵ {CH} symbolizes an organic substrate (dissolved
8 organic matter), serving as a precursor for di- and tribrominated methanes. For additional
9 details see the text. The waved blue line indicates the sea level. Included in this figure (see the
10 box to the right) is the formation of highly toxic cyanogen bromide BrCN from bromide and
11 hydrogencarbonate by the benthic diatom *Nitzschia cf pellucida*. This diatom employs a yet to
12 be identified haloperoxidase HPO.⁵⁶

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19 The reaction with dimethylsulfide is of some interest in as far as (CH₃)₂S is a main
20 constituent in sulfur cycling between the hydrosphere and the atmosphere. In the marine
21 environment, (CH₃)₂S is released as a metabolite of dimethylsulfoniopropionate,⁵⁷ which in
22 turn is produced in a reaction cascade from sulfate via hydrogen sulfide and methionine. The
23 atmospheric re-oxidation of (CH₃)₂S to dimethylsulfoxide and further to sulfuric acid/sulfate
24 helps to replenish the marine sulfate contents. Another interesting branching out is the
25 oxidation of atmospheric elemental mercury. Mercury is released into the atmosphere in the
26 frame of volcanism, anthropogenic activity and, from sea water and soils, after methylation of
27 inorganic mercuric compounds (Hg²⁺ → CH₃HgI → CH₃HgCl → (CH₃)₂Hg) and subsequent
28 volatilization and photolysis of the dimethylmercury ((CH₃)₂Hg → Hg + C₂H₆). As shown in
29 green in Fig. 6, bromine radicals can reoxidize Hg⁰ to Hg²⁺, which then becomes
30 rediposited.⁵⁸

41 5. Vanadium's potential role in medicinal applications

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46 In the introductory section 1, the similarity between vanadate and phosphate has been pointed
47 out (see e.g. Fig. 1), enabling vanadate to substitute for phosphate in phosphate-dependent
48 physiological processes, such as those depending on, or regulated by, phosphatases, kinases,
49 phosphomutases and -diesterases, ATPases and ribonucleases. The pharmacological activity
50 of vanadate in the amelioration of diabetic symptoms is closely related to this specific
51 interchange. The first clinical application of vanadate, in the form of aqueous solutions, in fact
52 goes back to the end of the 19th century.⁵⁹ In addition to vanadium's anti-diabetic (insulin-
53 enhancing) effect, vanadium compounds have been shown to have pharmacological activity in
54 the treatment of parasitic diseases, malign tumors, bacterial and viral infections. A plethora of
55 vanadium-based medications has been investigated in this respect, a selection of which can be
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found in recent reviews.^{8, 11} In most cases, oxidovanadium(IV) and –(V) coordination compounds with organic ligand systems coordinating through O-, N- and S-functions have been looked at, but a few traditional as well as more recent examples for the cancerostatic activity of organovanadium compounds, containing the π bonding cyclopentadienyl system, have also appeared (see below).

This section will be constrained to a brief treatise of a few selected examples, thus providing an overview of the inherent capability of vanadium compounds in coping with diseases. It should be noted, however, that so far vanadium compounds have not yet been introduced into actual medicinal applications. It should further be pointed out that vanadium compounds undergo at least partial biotransformation in the blood and other body fluids, i.e. the pharmaceutically active species commonly is not the same as the applied compound.⁶⁰

The most important transporter for H_2VO_4^- , VO^{2+} (and vanadium compounds with an accessible coordination site) in blood is serum transferrin Tf. VO^{2+} also binds to immunoglobulin and serum albumin, though less efficiently than to Tf. In addition, red blood cells contribute in the uptake, transport and subsequent distribution, provided that $\text{VO}^{2+}/\text{VO}_2^+$ is coordinated to an appropriate carrier, i.e. a ligand systems that is able to transcend cellular membranes.⁶¹ Inside the erythrocytes, VO_2^+ is reduced to VO^{2+} which is then partially released from the genuine “drug” and binds to, inter alia, haemoglobin Hb. However, Hb can also coordinate to the intact complex, likely via a histidine residue; an example is $\text{VO}(\text{maltol})\text{Hb}$. Other proteins can likewise stabilize intact vanadium complexes, as recently demonstrated for the coordination of the $\text{VO}(\text{picolinate})_2$ moiety to a carboxylate oxygen of a side chain aspartate of a lysozyme, Fig. 7.^{62a} Lysozymes are glycoside hydrolases. Picolinatovanadium complexes have otherwise intensively been studied for their insulin-enhancing properties in animal and laboratory models.^{62b, c}

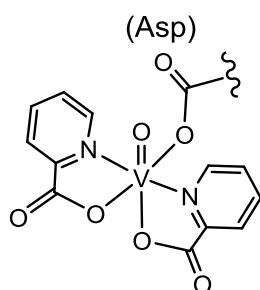


Fig. 7 Coordination of the $\text{V}^{\text{IV}}\text{O}(\text{pic})_2$ unit (pic = picolinate(1-)) to the aspartate residue Asp52 of hen egg white lysozyme.^{62b, c}

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First clinical trials with simple inorganic vanadium compounds in diabetic individuals have been performed in the last decade of the bygone century.^{63, 64} An advanced clinical study – clinical tests phase IIa – has been carried out more recently with the insulin-enhancing VO²⁺ maltolato complex BEOV = VO(ethylmaltol)₂(H₂O).⁷ A possible and simplified mode of action is illustrated in Fig. 8. Accordingly, BEOV undergoes (partial) speciation in blood serum. The speciation includes removal of the maltolato ligand, coordination of the VO²⁺ moiety to Tf, and/or oxidation to vanadate. Both the Tf complex and vanadate can enter the intracellular space via endocytosis and through phosphate channels, respectively. The insulin receptor IR is a trans-membrane receptor having at its disposal tyrosine residues linked to the intracellular β subunits. Docking of insulin to the extracellular α subunit promotes phosphorylation of the tyrosines. In the absence of insulin (type I diabetes) or in the case of insufficient insulin response of the receptor (common type II diabetes), a protein tyrosine phosphatase PTP counteracts the phosphorylation of IRβ and thus the signaling path (red arrows) responsible for the cellular uptake of glucose (mauve arrows) by the glucose transporter GLUT4. This is the point where vanadate comes in: vanadate strongly coordinates to a cysteine residue of the PTP, thus preventing dephosphorylation of the IRβ subunits and restoring the signaling path.

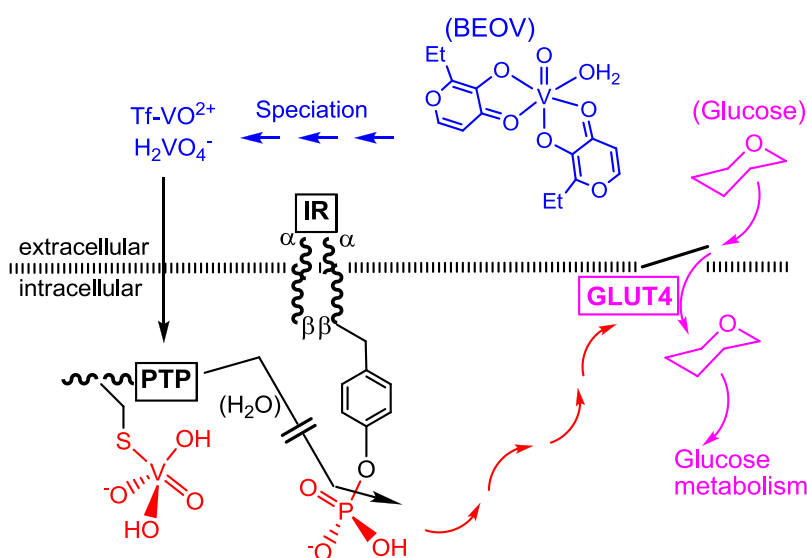


Fig. 8 A simplified illustration of the action of vanadate as an insulin-mimetic/enhancing agent (red and mauve traces). BEOV is *bis*(ethylmaltolato)oxidovanadium(IV); Tf = transferrin, IR = insulin receptor, PTP = protein tyrosine phosphatase. GLUT4 is a glucose transporter. For additional details, see the text.

Fig. 9 provides a compilation of examples of an increasing number of vanadium coordination compounds that display *in vitro* and/or *in vivo* activity against parasitic tropical diseases (**1**, **2** and **3**), bacterial (**4**) and viral (**5**) infections. The bis(peroxido)vanadium complex **1** is effective against the *Leishmania* flagellates, hence the protozoan parasites responsible for leishmaniasis,⁶⁵ a wide-spread disease that is transmitted by sandflies predominantly in tropical and subtropical areas. People infected by the *Leishmania* parasite suffer from cutaneous and visceral infections. The potentiality of compound **1** likely can be attributed to the formation of radicals such as superoxide and nitrous oxide. Compound **2** is a potential therapeutic tool to fight Chagas disease,⁶⁶ also known as American trypanosomiasis, and sleeping disease. Swollen lymph nodes as well as cardiac and digestive disorder are typical symptoms for Chagas disease. The disease is triggered by *Trypanosomas cruzi*, a protozoa transmitted by the kissing bug (*Triatoma infestans*).

Gastrointestinal infections and, in more serious cases, also liver abscesses go along with amoebiasis, brought about by the amoeba *Entamoeba histolytica*. The hydrazine complex **3** is more effective against the parasite than metronidazole, a common medication against amoebiasis.⁶⁷ Complex **4** in Fig. 9, with a thiosemicarbazone ligand linked to the dioxidovanadium(V) fragment features anti-tuberculosis activity,⁶⁸ and several oxidovanadium(IV) porphyrin complexes, **5** in Fig. 9,⁶⁹ have been shown to efficiently inhibit the human immunodeficiency virus (HIV) that is causative for AIDS. Antibacterial and antiviral activities have also been reported for polyoxidovanadates such as $[V_{15}O_{36}(CO_3)]^{7-}$.⁷⁰

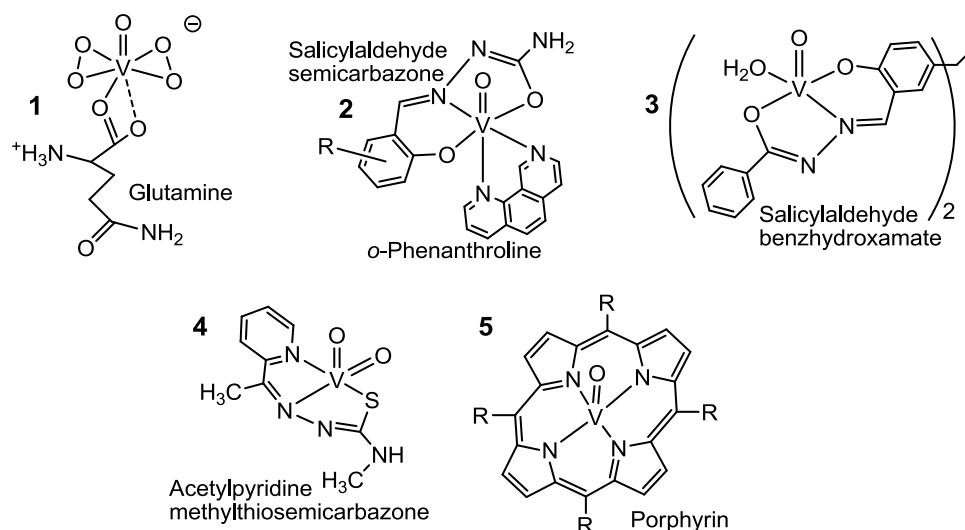


Fig. 9 Oxidovanadium complexes that are active against parasites causing tropical diseases such as leishmaniasis (**1**), Chagas disease and sleeping sickness (trypanosomiasis) (**2**),

amoebiasis (**3**), or fight bacterial infections (such as caused by *Mycobacterium tuberculosis*, compound **4**) and viral infections such as HIV (**5**).

Several of the compounds mentioned above, in particular the porphyrin complex **5** and the polyoxidovanadates, are also active in cancer treatment. More specifically, the complexes **6**, **7** and **8** in Fig. 10 exhibit anti-cancer activity. The flavonoid ligand silibinin in **6** has been isolated from extracts of the milk thistle (*Silybium marianum*); the corresponding vanadium complex inhibits the viability of human osteosarcoma cells.⁷¹ The nicotinoylhydrazone complex **7** features anti-cancer activity against cervical cancer,⁷² and the vanadocene derivative **8** is cytotoxic against renal cancer cells.⁷³ The cytotoxicity of compound **8** carries on a long-standing tradition in cancer research with titanocenes and vanadocenes, originally going back to Köpf and Köpf-Meier.⁷⁴ Finally, vanadium complexes have proven neuro-protective and cardio-protective effects. Examples are the *bis*(peroxido)-picolinato complex **9** in Fig. 10, and *bis*(maltolato)-oxidovanadium (Fig. 8). The maltolato complex attenuates myocardial reperfusion,⁷⁵ i.e. blood flow is restored to tissues that had their blood supply cut off, and complex **9** promotes neuroprotection, for example in the case of cervical spinal cord injury (spinal trauma).⁷⁶

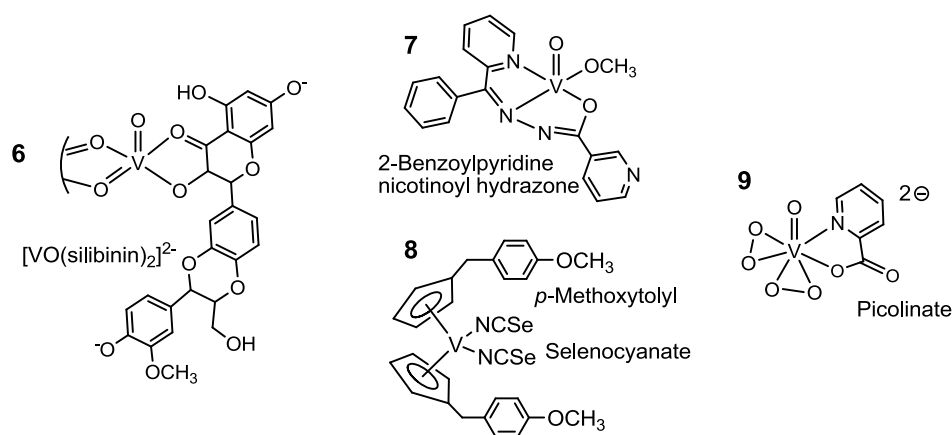


Fig. 10 A selection of vanadium compounds that exhibit an anti-cancer potential (**6**, **7**, **8**) or attenuate myocardial reperfusion (**9**). The structure of **6** has been deduced from EPR details provided in ref.⁷⁷

6. Concluding remarks

Along with the transition elements Mo, W, Mn, Fe, Co, Ni, Cu and Zn, vanadium is an essential bioelement, but in contrast to most of these element (namely Mo, Mn, Fe, Co, Cu and Zn), which are generally essential for all life forms, *functional* vanadium compounds have

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3 so far only been detected in the form of vanadium nitrogenases and vanadate-dependent
4 haloperoxidases in a comparatively restricted number of organisms. Further, some bacteria
5 may employ vanadium in nitrate reductases, and vanadate is used by various anaerobic
6 prokarya in respiration, commonly along with other transition metal compounds containing
7 the metal in a high oxidation state. A few groups of organisms – sea squirts, fan worms,
8 *Amanita* mushrooms – accumulate vanadium without an apparent benefit or, to formulate this
9 issue more cautiously, so far without an apparent reason why these organisms recur to
10 vanadium in its biologically rather unusual forms V^{III} (sea squirts and fan worms) and non-
11 oxido V^{IV} (amavadin).
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19 As far as the well-established vanadium-dependent nitrogenases and peroxidases are
20 concerned, their impact on nature is noteworthy: The vanadium nitrogenases of soil bacteria
21 such as *Azotobacter* and planctic cyanobacteria (*Anabaena*) contribute substantially to
22 nitrogen fixations, and hence to the supply of ammonium for the global plant growth, while
23 vanadate-dependent haloperoxidases in macroalgae are co-responsible for the supply of
24 methylhalides to the atmosphere and thus the regulation of ozone levels. In addition,
25 chloroperoxidases have a noteworthy potential in the defense against microbial spoliation.
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32 The omnipresence of vanadium in our food, in drinking water and the surroundings on
33 the one hand, and the striking similarity of vanadate and phosphate on the other hand (the
34 differences between the transition metal vanadium and the main group element phosphorus
35 notwithstanding), suggest that vanadium also attains a general role in life, in as far as
36 vanadate can interfere with phosphate in a variety of metabolic processes, reflected for
37 example by the dramatically increasing number of protein structures in which phosphate is
38 replaced by vanadate,⁷⁸ and by the fact that vanadate – and simple vanadium compounds as
39 likely precursors for vanadate – can ameliorate the outcome of diabetes mellitus.^{7,79} The
40 phosphate-vanadate antagonism, as well as the specific properties of vanadium coordination
41 compounds implemented by the ligand system, have increasingly motivated working groups
42 to explore and to fathom the potentiality of vanadium complexes in combatting parasitic
43 (tropical) diseases, bacterial and viral infections, but also in the treatment of various bodily
44 dysfunctions, such as uncontrolled cell growth (cancer), cardio-vascular and neuronal
45 problems.
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56 To date, little resilient detail is known about the perspective essentiality of vanadium
57 and its handling in higher organisms, a fact that should encourage enhanced research activities
58 directed towards, for example, vanadium's role in the treatment of the diseases and bodily
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dysfunctions sketched above. Similar considerations apply to the mechanisms of action of unphysiologically high exposure to vanadium and its concomitant (potential) toxicity.^{4b}

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