Is vanadium a more versatile target in the activity of primordial life forms than hitherto anticipated?

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The two predominant forms of vanadium occurring in the geo-, aqua- and biosphere, soluble vanadate(V) and insoluble oxovanadium(IV) (vanadyl), are subject to bacterial activity and transformation. Bacteria belonging to genera such as *Shewanella*, *Pseudomonas* and *Geobacter* can use vanadate as a primary electron acceptor in dissimilation or respiration, an important issue in the context of biomineralisation and soil detoxification. *Azotobacter*, which can employ vanadium as an essential element in nitrogen fixation, secretes a vanadophore which enables the uptake of vanadium(V). Siderophores secreted by other bacteria competitively (to ferric iron) take up vanadyl and thus interfere with iron supply, resulting in bacteriostasis. The halo-alkaliphilic *Thioalkalivibrio nitratireducens* possibly uses vanadium as a constituent of an alternative, molybdopterin-free nitrate reductase. Marine macro-algae can generate a variety of halogenated organic compounds by use of vanadate-dependent haloperoxidases, and a molecular vanadium compound, amavadin, from *Amanita* mushrooms has turned out to be an efficient catalyst in oxidation reactions. The present account is a focused and critical review of the current knowledge of the interplay of bacteria and other primitive forms of life (cyanobacteria, algae, fungi and lichens) with vanadium, with the aim to provide perspectives for applications and further investigations.

Introduction

With a concentration of *ca.* 30 nM, vanadium is the second-to-most abundant transition metal in sea-water, exceeded only by molybdenum (100 nM), and clearly more abundant than iron ($\leq$0.7 nM). Vanadium is mainly present in its easily soluble form, dihydrogen-orthovanadate(V), ion-paired to sodium ions, *viz.* $Na^+H_2VO_4^-$. Vanadium is also omnipresent in soils and fresh water reservoirs in its soluble (vanadate(V)) and insoluble form (mainly oxovanadium(IV) = vanadyl VO^{2+}), in the latter case absorbed to particulate matter or mobilised by "vanadophores" (*vide infra*). Riverine input of vanadium to the oceans is partially removed by sea-floor vent-derived iron oxides, controlling the concentration and cycling of vanadium.[1]

Vanadium is thus generally available for living organisms. The two predominant vanadium forms are redox coupled ($H_2V^VO_4^-/V^{IV}O^{2+}$) with a redox potential $E^{pH=7} = -0.34$ V (*vs.* NHE), well within the range of physiological redox processes. Marine organisms such as sea-squirts (*Ascidiaceae*)[2] and the fan worm *Pseudopotamilla occelata*[3] take up vanadate from sea water, reduce it to vanadyl, which is intermittently bound to vanadium-binding proteins particularly rich in lysine (**1** in Fig. 1), and finally further reduced to vanadium(III) and stored in specialised blood cells (vanadocytes) in concentrations up to 0.3 M. Many marine macro-algae, *e. g.* the brown alga *Ascophyllum nodosum*[4a] and the red alga *Corallina pilulifera*[4b] contain a haloperoxidase in which vanadate is covalently linked to a histidine of the protein matrix (**2** in Fig. 1). These haloperoxidases are also present in certain terrestrial fungi and lichens. Finally, the fly agaric (*Amanita muscaria*) contains an organic non-oxo vanadium(IV) compound, amavadin, apparently without any function, in which vanadium is coordinated to two dichiral, trianionic ligands derived from *N*-hydroxyimino-2,2′-diisopropionic acid (H_6hidpa), **3** in Fig. 1.[5]

Nitrogen-fixing bacteria (*Azotobacter vinelandii* and *A. chroococcum*) and cyanobacteria (*Anabaenae*) employ vanadium as an alternative to molybdenum in the absence of the latter, or at low temperatures, as a constituent of nitrogenase (**4** in

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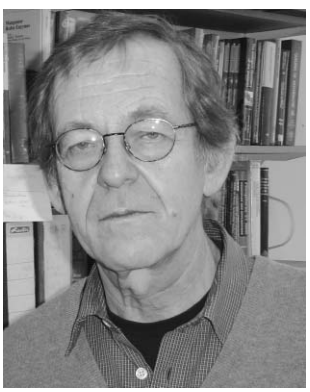

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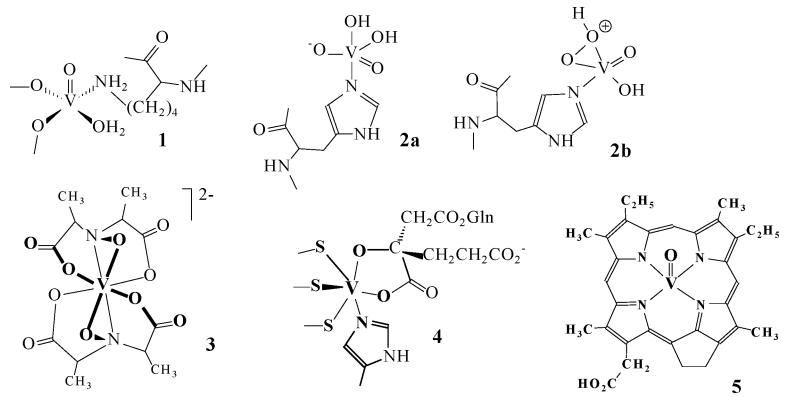

Fig. 1 Naturally occurring vanadiumorganic compounds. **1**: Proposed structure for vanadyl coordinated to a lysine side-chain of vanabin, a vanadium binding protein from the sea squirt *Ascidia sydneiensis samea*; **2**: the active centre of vanadate-dependent haloperoxidases (**2a**) and its active hydroperoxo form (**2b**); **3**: $[\Delta\text{-VO}\{S,S\text{-(hidpa)}\}_2]^{2-}$ (amavadin) from *Amanita muscaria*; **4**: the immediate vanadium environment in the *M* cluster of vanadium-nitrogenase from *Azotobacter vinelandii*; **5**: vanadyl-desoxiphyllerythrine, a vanadium compound present in crude oil.

Fig. 1).[6] While this specific bacterial use of vanadium is well established, there have been reports during the last two decades on other missions of vanadium in bacterial function, metabolism and growth which, when established, can be of considerable interest as it comes to the detoxification of, *e. g.*, ground water in areas impacted by mining activities, or in other environmental issues related to vanadium overload. Understanding these bacterial functions can also help to cast light on the (geochemical) cycling of vanadium, including its conversion to vanadiumorganic compounds such as the porphinogenic system **5** in Fig. 1, present in crude oils, oil shales and the like, and in understanding the role of microbial and cellular speciation of vanadium, a problem which has previously been addressed with the aid of the fungus *Saccharomyces cerevisiae.*[7]

Bacterial use and speciation of vanadium will be dealt with in the context of vanadium in a putative vanadium-dependent nitrate-reductase,[8] the role of siderophores in mobilising vanadium, including a vanadophore from *Azotobacter*,[9] and the use of vanadate(V) as primary electron acceptor in bacterial respiration, in particular by the soil bacterium *Shewanella oneidensis.*[10]

An alternative nitrate reductase?

Nitrate reductases are enzymes that commonly catalyse the two-electron reduction of nitrate to nitrite; eqn (1). They regularly contain molybdenum as a constituent of a so-called molybdopterin cofactor, in which Mo (changing between the oxidation states +IV and +VI) is coordinated to a dithiolene moiety of a tetrahydropterin derivative. Nitrate reductases lacking Mo and this specific cofactor, and containing vanadium instead, have been isolated and characterised from the halo-alkaliphilic sulfur bacterium *Thioalkalivibrio nitratireducens*,[8] and the facultatively anaerobic, chemolithotrophic bacterium *Pseudomonas isachenkovii.*[11,12] The respective enzyme from *T. nitratireducens* is a homotetramer of molecular mass 195 kDa, which contains vanadium and iron in a molar ratio 1 : 3, along with haeme-*c*. Energy source for the nitrate reduction is thiosulfate. *In vitro*, this enzyme also promotes the reduction of nitrite (to N_2O; eqn (2)), chlorate, bromate, selenate and sulfite. It further exhibits a haloperoxidase activity, *i.e.* it catalyses the oxidation of halide X^- to an $\{X^+\}$ species, *e. g.*, hypohalous acid, using hydrogen peroxide as oxidant; eqn (3). Whether the haloperoxidase activity is associated with haeme-*c* (and thus related to haeme-type peroxidases) or the presence of vanadium (*i.e.* related to vanadate-dependent peroxidases as in marine algae; *vide infra*) has not been revealed. An enzyme exhibiting activity in reduction *and* oxidation certainly is an unusual feature, although compatible with the chemistry of vanadium, which easily changes between the oxidation states +V, +IV and +III.

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O \quad (1)$$

$$2NO_2^- + 4e^- + 6H^+ \rightarrow N_2O + 3H_2O \quad (2)$$

$$X^- + H_2O_2 + H^+ \rightarrow HXO + H_2O\ (X = Cl, Br, I) \quad (3)$$

The pterin cofactor is also lacking in the alternative, periplasmatic nitrate reductase from *P. isachenkovii*, nor does the enzyme from this source contain a haeme-*c*, presupposing that vanadium is directly coordinated to side-chain functions of the protein, *e. g.* to the amine nitrogen of histidine, as in the case of vanadate(V)-dependent peroxidases (**2** in Fig. 1), or of lysine, glutamine or arginine, as suggested for VO^{2+} in vanabins from ascidians (**1** in Fig. 1). *P. isachenkovii*, when grown in a culture medium containing lactate as an electron donor and vanadate(V) as primary electron acceptor secretes a protein (molecular mass 14 kDa) into the culture medium which coordinates up to 20 vanadyl ion per protein,[13] resembling, in this respect, the vanabins, and possibly classifying it as a storage protein. The synthesis of a vanadium(IV)-binding protein and its secretion may be a way of detoxification of excessive vanadate (vanadate is an effective inhibitor of ATPases, kinases and ribonucleases).

The presence of a periplasmatic vanadium-containing nitrate/vanadate reductase, on the other hand, appears to indicate that *P. isachenkovii* also employs vanadium in its dissimilatory functions. The reductase, a tetramer, has a molecular mass of 220 kDa, an activity optimum at 80°, and a pH optimum of 6.0–8.5. Nitrate is reduced to dinitrogen. Vanadate reduction starts only after denitrification has been almost completed. On the other

hand, for *Shewanella* it has been demonstrated (see below) that the reduction of vanadium(V) does *not* depend on the induction of specific proteins.

Vanadate as a primary electron acceptor

Vanadate reduction to primarily vanadyl, eqn (4), has also been noted for *Pseudomonas vanadiumreductans*[14] and *Geobacter metallireducens* ("feeding" on acetate),[15] and investigated thoroughly for *Shewanella oneidensis*.[10] The *Pseudomonas* bacteria, which also generate some vanadium(III), eqn (5), can use several organic electron donors (sugars, organic acids), but also carbon monoxide and dihydrogen as electron source. The vanadium deposits formed by *P. vanadiumreductans*, containing both V^{V} and V^{IV}, resemble the vanadium mineral sherwoodite, $Ca_9Al_2V^{IV}{}_4V^{V}{}_{24}O_{80}\cdot 56H_2O$, casting some light on the possible role of these (and other) bacteria in biomineralisation. Vanadate respiration by *G. metallireducens* has been shown to be an effective strategy to decontaminate subsurface environments burdened with vanadium from industrial and mining activities, or natural sources.[15]

$$H_2VO_4^- + e^- + 4H^+ \rightarrow VO^{2+} + 3H_2O \quad (4)$$

$$VO^{2+} + e^- + 2H^+ \rightarrow V^{3+} + H_2O \quad (5)$$

S. oneidensis, renowned for its respiratory versatility, is able to use over twenty terminal electron acceptors, including Fe^{3+}, Mn^{4+}, U^{6+}, MoO_4^{2-} and $H_2VO_4^-$. Reduction of vanadate(V) by *S. oneidensis*, employing lactate as the electron donor,[16] clearly is *respiratory* (proper culture conditions provided[10]) *i.e.* coupled with proton translocation, ATP formation and consequently growth (dissimilatory reduction occurs without the generation of a proton motive force). The average biomass doubling time amounts to *ca.* 10 h; typical reduction rates are 12 mmol vanadium per h and g of cells. The biomass yield is proportional to the amount of vanadate used. The product of reduction is VO^{2+}, which provides a bluish colouration to the growth medium. A model for the electron transfer pathway, coupled with proton delocation, ATP formation and outer membrane vanadate reduction,[10] supported by investigations of the reduction of Fe^{III} to Fe^{II},[17] is depicted in Fig. 2.

In this simplified model, lactate has been chosen as the primary electron source which, by action of a dehydrogenase, delivers protons on conversion to pyruvate. It has been shown that menaquinones (and not ubiquinones) play a crucial role in further dealing with the reduction equivalents: mutants of *S. oneidensis* lacking the genes for menaquinone and CymA are severely limited in vanadate reduction.[18] CymA, a tetrahaeme *c*-type cytochrome associated with the periplasmatic inner membrane, acts as a menaquinol dehydrogenase. The proton gradient (and the electrochemical gradient) generated by these redox processes are then exploited to store energy in the form of ATP. Of interest in this context is the observation that the proton-translocating ATPase from the bacterium *Vibrio parahaemolyticus*—another vanadate reducer—is not at all inhibited by vanadate,[19] a remarkable issue since vanadate otherwise is a very effective inhibitor of ATPases. In the reduction of ferric iron, the next step in electron shuttle is achieved by periplasmatic decahaeme cytochromes (MtrD and MtrA).[17] It is likely that these cytochromes are also involved in vanadate reduction. At least some of the vanadate appears to be reduced in the periplasmatic space, as revealed by transmission electron microscopy, showing the formation of granules located in the periplasm, possibly representing insoluble vanadylhydroxides.[20] The main amount of vanadate is reduced by the outer-membrane decahaeme *c*-type cytochromes MtrC/OmcB; *omcB*-deficient mutants reduce vanadate only to a very limited extent. The extracellular reduction product, blue soluble VO^{2+} (stabilised by ingredients of the culture medium or excretions of the bacterial cells ligating to the vanadyl ion) in the first instance, converts to a granular precipitate consisting of vanadylhydroxides and condensation products thereof, colonised by the bacteria.

Rusty brown to blackish precipitates have also been observed in the course of anaerobic vanadate reduction by bacteria associated with and isolated from deep sea hydrothermal vent worms.[21] The colouration, and hence the composition, of the reduction products depends on the nature of the applied vanadate, *viz.* orthovanadate *vs.* metavanadate. The reasons for these differentiations are not quite clear. They might reflect influences of the pH: solutions prepared from metavanadate are neutral, eqn (6a), solutions of orthovanadate are alkaline, eqn (6b). In either case, these reactions are succeeded, in the concentration range applied (5–8 mM),

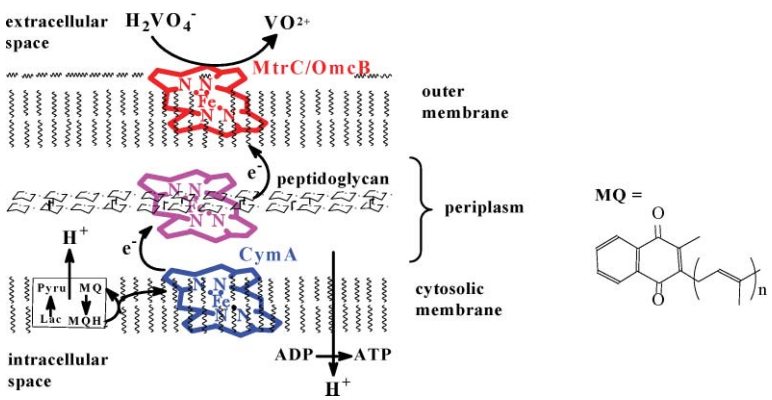

Fig. 2 Model for the electron pathway from the inner membrane to the vanadate reductase (OmcB) located at the outer membrane of *S. oneidensis* strain MR-1. The gap between CymA (a tetrameric haeme-type protein) and MtrC/OmcB (decameric haeme proteins) is surpassed by periplasmatic cytochromes. MQ/H_2MQ = menachinone/-chinol.

by condensation to di- and tetravanadate; see, *e.g.*, the non-stoichiometric eqn (6c). The culture medium should, however, equilibrate pH influences.

$$VO_3^- + H_2O \rightarrow H_2VO_4^- \quad (6a)$$

$$VO_4^{3-} + H_2O \rightarrow OH^- + HVO_4^{2-} \quad (6b)$$

$$H_2VO_4^- \rightleftarrows H_2O + H_2V_2O_7^- \text{ and } V_4O_{12}^{4-} \quad (6c)$$

Vanadate is also reduced to vanadyl by wild type and respiratory-deficient strains of beer yeast (*Saccharomyces cerivisiae*). Contrasting *Shewanella*, vanadyl is accumulated in the cell, *i.e.* medium vanadate, at concentrations of 1 mM (where growth is not inhibited), is converted to cellular vanadyl.[7a] Cytoplasmatic glutathion and catechols have been proposed as reductants, in accordance with the known chemistry of vanadium(V). At considerably higher, growth-inhibiting concentrations efflux of vanadyl from the cells is observed along with cell associated vanadyl species. As demonstrated by EPR spectroscopy, dinuclear oxovanadium units are present.[22]

Vanadium-chelating siderophores and vanadophores

Siderophores (Greek for "iron-carriers") are ligand systems secreted by, *inter alia*, bacteria. Siderophores have characteristics enabling them (i) to efficiently coordinate ferric ions and (ii) to transport the iron thus mobilised from insoluble deposits through an aqueous medium before being incorporated by the cell. This is a vitally necessary strategy for organisms living in aerobic compartments where iron only exists in the form of practically insoluble ferric hydroxides/oxides. As shown in the previous chapter, vanadium can become unavailable when reduced to VO^{2+}, where it forms sparingly soluble vanadyl hydroxides/oxides under anoxic conditions, and still remains in this insoluble V^{IV} state when conditions turn back to oxic, competing with iron for the siderophores and thus preventing sufficient supply of the organisms with iron.

Two siderophores that have been well investigated are deferoxamine (H_4df^+, also termed desferrioxamine) and enterobactin H_6ent, secreted by actinobacteria belonging to the genus *Streptomyces*, and by proteobacteria such as the enteric *Escherichia coli*, respectively. Deferoxamine is an iron chelator which is also being employed to treat iron overload such as thalassaemia. Both siderophores, H_4df^+ and H_6ent, have been shown to also effectively coordinate vanadium in its oxidation state +IV. In the case of the hydroxamic acid deferoxamine B, a complex of the likely composition $[VO(OH)(H_2O)_2H_2df]$ (**6** in Fig. 3) is formed under slightly acidic conditions,[23] eqn (7), with an apparent formation constant at pH 4 of $K_{app} \approx 10^7$ M^{-1}. In this complex, the siderophore uses only two of its three hydroxamate functions for coordination to the vanadyl centre. Enterobactin H_6ent, which contains a *tris*serine lactone back bone and three catechol functionalities, forms the non-oxo vanadium(IV) complex $[V(ent)]^{2-}$, eqn (8) and **7** in Fig. 3.[24] Elimination of the oxo group in VO^{2+} is a feature commonly observed with catecholato ligands.

$$[VO(H_2O)_4(OH)]^+ + H_4df^+ \rightleftarrows [VO(H_2O)_2(OH)(H_2df^+)]\ (\mathbf{6}) + 2H^+ + H_2O \quad (7)$$

$$[VO(H_2O)_4(OH)]^+ + H_6ent \rightleftarrows [V(ent)]^{2-}\ (\mathbf{7}) + 3H^+ + 6H_2O \quad (8)$$

The competition of vanadium with iron for binding by siderophores is evident for the strong growth inhibition of

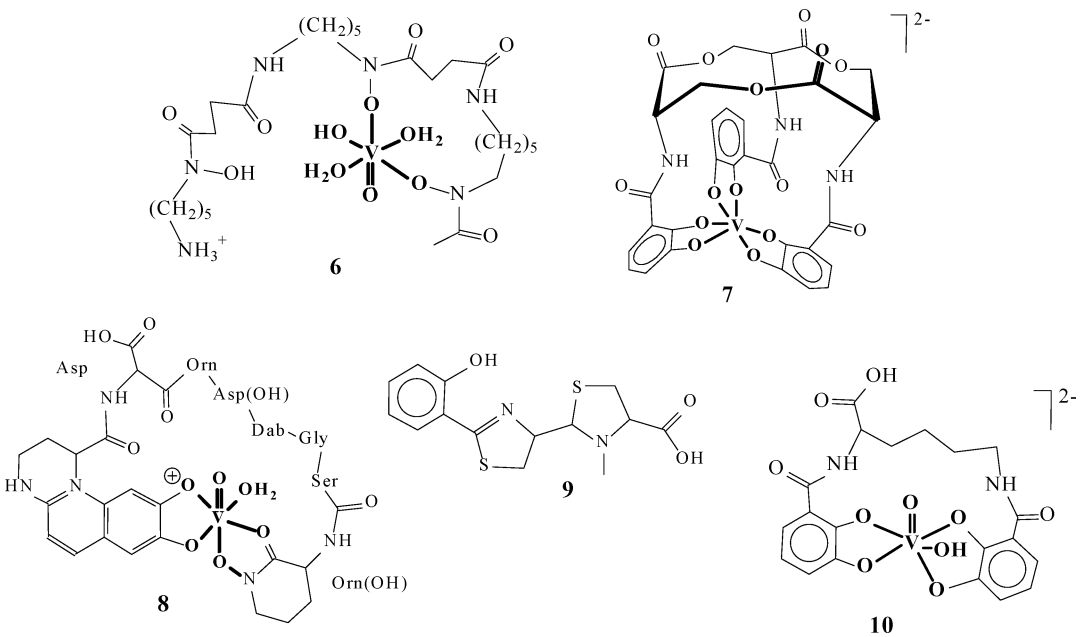

Fig. 3 Vanadium complexes built with the siderophores deferoxamine B (**6**, containing $V^{IV}O^{2+}$), enterobactin (**7**, containing V^{IV}), pyoverdine (**8**, containing $V^{IV}O^{2+}$) and azotochelin (**10**, containing $V^{V}O^{3+}$). The siderophore **9**, pyochelin, can form ligand–vanadium 2 : 1 complexes with $V^{IV}O^{2+}$ and with vanadate(V). **6** and **8** are proposed structures, **7** has been structurally characterised by X-ray diffraction, and the structure of **10** is based on the structural characterisation of the related molybdenum(VI) complex. In the pyoverdine represented in **8**, the chromophore carries a peptide chain made up of seven amino acids: Asp(OH) = hydroxyaspartic acid, Dab = 2,4-diaminobutyric acid, Orn(OH) = *N*-hydroxyornithine.

Pseudomonas aeruginosa on addition of 1–2 mM vanadyl to the culture medium.[25] *P. aeruginosa* produces two siderophores under iron-limiting conditions, pyoverdine H_3pvd^+, and the thiazoline derivative pyochelin, H_2pch. Pyoverdine consists of a chromophore, a chinoline derivative which provides two catechol functions for coordination to a metal centre, and a peptide chain, terminated by *cyclo*-OH-ornithine, furnishing two additional coordination sites as shown for [VO(pvd)] (**8**) in Fig. 3. Evidence for this coordination mode comes from MAS spectra.[25] Pyochelin, **9** in Fig. 3, forms an anionic vanadium–ligand 1 : 2 complex ($[VO(pch)_2]^{n-}$) with both, V^{IV} ($n = 2$) and V^{V} ($n = 1$), where coordination probably takes place through the deprotonated phenol and carboxylic acid functions. The complex has a strong antibacterial effect. The conversion of the V^{IV} to the V^{V} form can generate superoxide, eqn (9), and thus give rise to oxidative stress.

$$[VO(pch)_2]^{2-} + O_2 \rightarrow [VO(pch)_2]^{-} + O_2^{-} \quad (9)$$

In the context of oxidative stress, it is of interest that mutants of *Pseudomonas fluorescens* with transposons inserted in genes coding for the tricarboxylic acid cycle exhibit resistance to vanadium. Citrate is isomerised by aconitase to isocitrate (*cf.* Fig. 4). Of the three aconitase genes, *acnD* is switched off in the 22C3 mutant. This specific gene encodes [Fe–S] dependent activity ([Fe–S] stands for an iron-sulfur cluster), which is particularly sensitive towards reactive oxygen species. Its deactivation initiates overproduction of the two other aconitases (AcnA and AcnB) which are less sensitive or insensitive to oxidative stress. In addition to the 22C3 mutant deactivating the aconitase gene *acnD*, 7D9 insertion into the gene *idh*, encoding NADP-dependent isocitrate dehydrogenase, also effectuates vanadium resistance.[26]

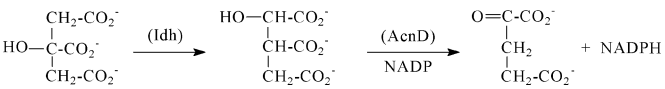

Fig. 4 Part of the citrate–isocitrate pathway in *Pseudomonas.* The *acnD* mutant 22C3 and the *idh* mutant 7D9 of *P. fluorescens* (strain ATCC 17400) show resistance against vanadyl sulfate.

With azotochelin, H_5ach, the V^{V} complex is exclusively formed. Azotochelin, produced by the free-living nitrogen-fixing bacterium *Azotobacter vinelandii*, acts as a siderophore, molybdophore and vanadophore.[9,27] *A. vinelandii* is able to reduce dinitrogen to ammonium ions (coupled with the reduction of protons to hydrogen, eqn (10), and energy-driven by ATP hydrolysis), employing a nitrogenase which relies on molybdenum or, alternatively, on vanadium. Azotochelin contains two catechol moieties linked by a lysine, which can take up ferric ions, molybdate and vanadate and thus increase the bioavailability of these metals, the acquisition of which is essential for diazotropic growth of the bacterium. The stability of the vanadium(V) complex, $[VO(OH)(Hach)]^{2-}$ (**10** in Fig. 3), or the corresponding protonated (aqua) or deprotonated (dioxo) form, quantified by the apparent formation constant $K_{app} = 6.3 \cdot 10^8\ M^{-1}$ (at pH. 6.6), surmounts that of the molybdenum(VI) complex by an order of magnitude. Under Mo-limiting conditions, vanadate triggers secretion of azotochelin; the vanadium complex then formed is taken up by the bacteria *via* a specific transport system, regulated by the concentration of vanadium.[27]

$$N_2 + 14H^+ + 12e^- \rightarrow 2[NH_4]^+ + 3H_2 \quad (10)$$

Oxidation catalysis by haloperoxidases and amavadin

Vanadate-dependent haloperoxidases from marine algae are responsible for a variety of halogenated organic compounds found in marine environments.[28,29] Bromoperoxidases (VBrPO) can catalyse the generation of simple compounds such as bromoform, and more complex ones, such as the two diastereomeric 8-epicapparapi oxides **12a** and **12b** in Fig. 5, generated from the sesquiterpene (*E*)-(+)-nerolidol, **11**.[30] This reaction is reminiscent of the laboratory synthesis of brominated pyrane and tetrahydrofuran derivatives (**14** and **15**) through reaction between the alkenol **13** and pyridinium hydrobromide and *tert*-butylperoxide, catalysed by the Schiff base vanadium complex **16**. Complex **16** has a coordination environment reminiscent of that in VBrPO (*cf.* **2** in Fig. 1).[31] Vanadate itself can catalyse oxidation reactions; a simple example, the oxidation of cyclohexane to cyclohexanol and cyclohexanone, is represented by the reaction scheme **17** in Fig. 5.[32] Another intriguing parallel between reactions catalysed by VBrPO and a vanadium catalyst, respectively, is the enantioselective formation of chiral sulfoxides from prochiral sulfides, as shown in Fig. 5 for the oxidation of thioanisol catalysed by VBrPO from different algal sources (the brown alga *Ascophyllum nodosum vs.* the red alga *Corallina pilulifera*),[33] or by the catalyst **18**,[34] and the oxidation of *p*-chlorothioanisol, employing the chiral catalyst system **19**.[35] Chiral sulfoxides are important synthons in organic chemistry, and in pharmaceutics (Sulindac®).

As noted earlier, the role of amavadin (**3** in Fig. 1) from *Amanita* mushrooms such as *A. muscaria* (the fly agaric) is elusive. Interestingly, amavadin and model compounds thereof catalyse oxidation and oxygenation reactions. The reaction represented by **17** in Fig. 5 is also catalysed by (synthetic[36]) amavadin with H_2O_2 as the oxidant. Bromocyclohexane is obtained if bromide is additionally present,[37] hence a catalytic activity comparable to that of VBrPO. Of considerable interest is the oxidation by peroxodisulfate of carbon monoxide, and in particular methane which is not easily activated, to carbonic acids with amavadin-based catalysts; see the non-stoichiometric eqns (11) and (12).[38] In the light of the diagonal relationship between molybdenum and vanadium, and the well established role of molybdenum in oxygenases and deoxygenases, it is attractive to assume that amavadin has been a component of a primitive redox-active enzyme, a remainder of, *e.g.*, an evolutionary overcome oxygenase;[39] Fig. 6. As in oxovanadium compounds, the vanadium oxidation state in the non-oxo compound amavadin easily changes between +IV (in the natural product) and +V.

$$CH_4 + S_2O_8^{2-} \rightarrow CH_3\text{-}CO_2H \quad (11)$$

$$RH + CO + S_2O_8^{2-} \rightarrow R\text{-}CO_2H \quad (12)$$

Conclusions and outlook

The availability of vanadium in sea water (second-to-most abundant transition metal), its general ubiquity along with its amphoteric character (anionic as vanadate, cationic in the form of nonoxo-, oxo- and dioxovanadium), and the ease with which it changes oxidation states under physiological conditions suggests that this element has already been used as an essential metal in the early stages of evolution. In Fig. 7, a phylogenetic tree is

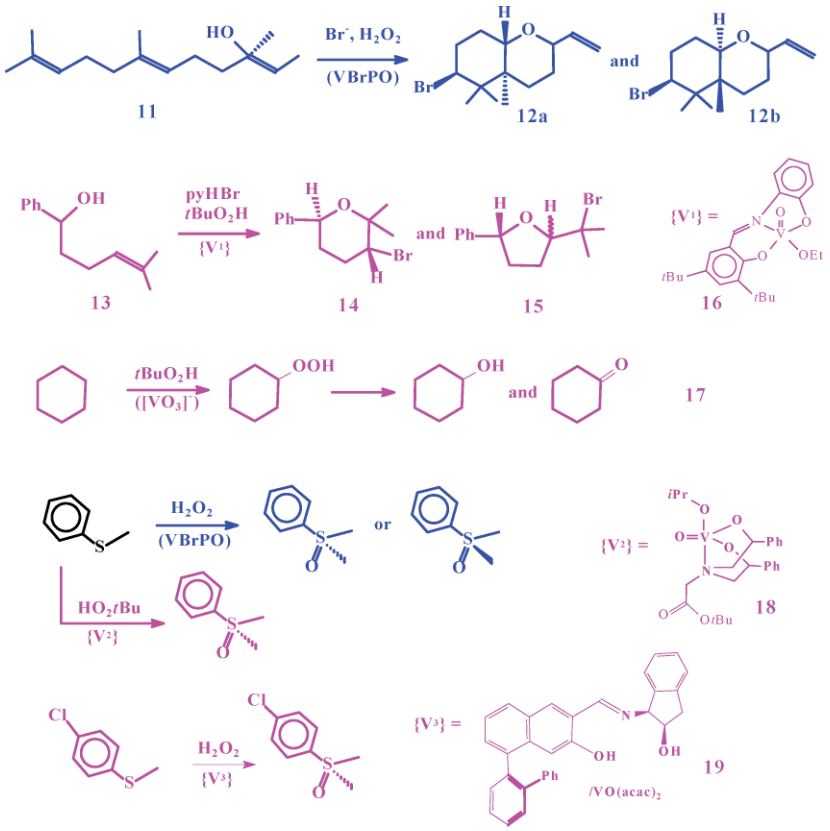

Fig. 5 Reactions that exemplify parallels between the synthetic potential of algal vanadate-dependent haloperoxidases (VBrPO) on the one hand, and inorganic vanadium catalysts on the other hand. Naturally occurring reactions are indicated in blue, model reactions in magenta.

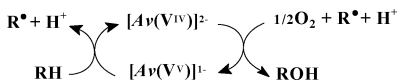

Fig. 6 Model for the oxygenase activity of a putative enzyme with amavadin (*Av*) in its active centre, adapted and modified from ref. [39]. For the structure of amavadin see **3** in Fig. 1.

shown, incorporating those organisms for which vanadium is—or can be—essential, spanning from cyanobacteria (approximately 3 billion years old) to green algae (*Chlorophyta*; *ca.* 0.8 billion years old).

The existence of an alternative, *i.e.* a vanadium-dependent nitrogenase in the proteobacterium *Azotobacter* and the cyanobacterium *Anabaena*, and a putative nitrate-reductase in

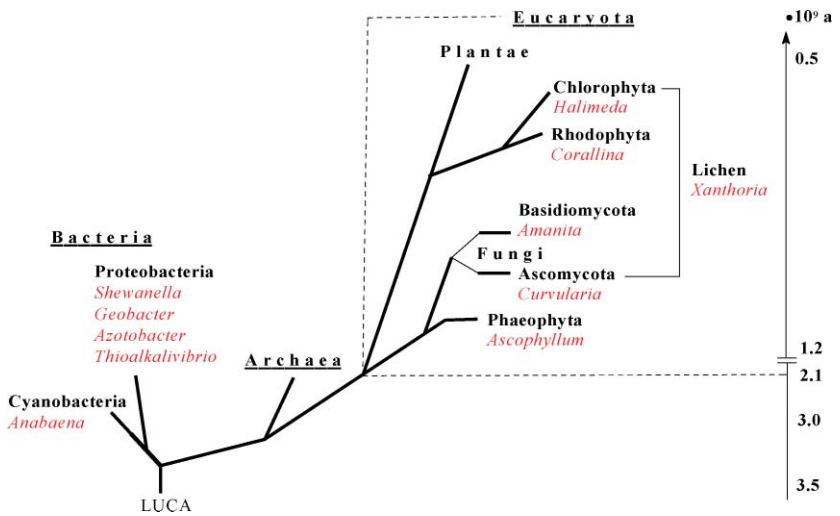

Fig. 7 Section of the phylogenetic tree, incorporating organisms, in red, that (can) use vanadium. All of these organisms belong either to the domains bacteria or eucaryotes; for archaea, functions of vanadium have so far not been reported. LUCA = last universal common ancestor. The time scale is approximate and not linear.

Thioalkalivibrio nitratireducens from soda lakes, are current examples for the (bacterial) use of vanadium, as are the vanadate-dependent peroxidases, widespread mainly in brown and red marine algae (*Phaeophyta* and *Rhodophyta*), but also present in certain green algae (*Halimeda* sp.), fungi (*Curvularia inaequalis*) and lichens (*e.g. Xanthoria parietena*, a symbiosis between an ascomycete and the green alga *Trebouxia*).[40] The same motif as in the (algal) vanadate-dependent peroxidases is present in vanadate-inhibited acid phosphatases from the pathogenic bacteria *Shigella flexneri* and *Salmonella enterica*, and these actually do exhibit peroxidase activity, though by an order of magnitude less pronounced than with the genuine algal peroxidases.[41] The similarities extend to conserved domains in the amino acid sequence of bacterial acid phosphatases and the vanadium peroxidases,[41] an intriguing feature that extends to the active site structure of the non-specific acid phosphatase from *Escherichia blattae*.[42]

The high abundance of vanadium in fossil matter of animal and plant origin prompts the question whether this element was formerly more intensively used by living organisms. Iron-only nitrogenases and haeme-type peroxidases are well established in nature along with the vanadium enzymes of comparable function; the competitive use of siderophores/vanadophores (piochelin from *P. aeruginosa*; azotochelin from *A. vinelandii*), and the use of vanadate(V) *or* Fe^{3+} as an electron acceptor by *S. oneidensis* may hint towards points of intersection in vanadium and iron metabolism. Additionally, intersections between vanadate and molybdate metabolism are conceivable. The chemistry of vanadium and molybdenum is similar to some extent in consequence of the diagonal relationship between these two elements. In the context of this report, the fact is noteworthy that molybdate(VI) can also energise respiration by *S. oneidensis*; and azotochelin from *A. vinelandii* also works as a molybdophore. Molybdenum-nitrogenase is the more common nitrogenase used by nitrogen-fixing bacteria and cyanobacteria, and molybdenum is a well established essential element in molybdopterin-based nitrate-reductase and other oxidases/reductases. The ability of the natural non-oxo vanadium compound amavadin from the fly agaric to catalyse oxygenation reactions *in vitro* certainly is an interesting fact in this context.

After all, vanadium appears to be a generally essential trace element for most if not all living organisms[40] insofar as it regulates, based on the similarity between vanadate $H_2VO_4^-$ and phosphate HPO_4^{2-}, phosphate-metabolising enzymes. Also from *this* point of view, more intensive investigations into the metabolism and function of elemental processes are desirable, involving vanadium in the simplest and oldest organisms on our planet, the domains bacteria and—unexplored as far as vanadium is concerned—archaea.

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