A Role for Vanadium in Ascidians and in Marine Algae

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INTRODUCTION

Vanadium is – next to molybdenum – the second-to-most abundant transition metal in sea water. Oxygenated sea water commonly contains 24-45 µM of vanadate $H_2VO_4^-$ (and is thus – next to molybdenum – the second-to-most abundant transition metal in sea water) with the levels mainly fluctuating with the season. Depletion by about 60% can occur as reduction to $V^{IV}O^{2+}$ takes place which forms a sparingly soluble hydroxide, $VO(OH)_2$, that is readily absorbed by particulate organic matter [1]. Consequently, the factors influencing the occurrence of vanadium are redox conditions (such as dissolved O_2 and Fe^{2+}, the presence of NH_3 and S^{2-}), and – of course – its uptake by marine organisms. Vanadate is mainly taken up by marine algae, the most prominent one being knotted wrack (also known as rockweed) *Ascophyllum nodosum*, (Figure 1), by ascidians and, to some extent, also by some *Polychaeta* fan worms [2]. The significance of vanadium as an essential element in these organisms will be addressed.

VANADIUM IN ASCIDIANS

In 1911, Henze discovered vanadium in the blood cells (the coelemic cells; singlet ring cells and vacuolated amoebocytes) of the Mediterranean sea-squirt (ascidian) *Phallusia mamillata* [3]. The vanadium compound present in these cells actually is hydrated vanadium in the oxidation states of (predominantly) high-spin +III, such as $V^{3+}(H_2O)_x$; the counter ion commonly is $H_2SO_4^-$. Vanadium


Figure 1: Examples for algae that produce hypohalous acids: Left *Corallina officinalis*, right *Ascophyllum nodosum*, the alga where the vanadate-dependent haloperoxidase was originally characterized by Vilter in 1983.

is taken up by the ascidians (Figure 2) in the form of vanadate. After uptake, it migrates – via phosphate channels – into the cytoplasm of the organism in the form of $H_2VO_4^-$. Concomitant reduction takes place, which apparently occurs in two steps, i.e. (1) reduction of vanadate (V) $H_2VO_4^-$ to oxidovanadium (IV) VO^{2+} by $NADPH^+$ and (2) reduction of VO^{2+} to (hydrated) V^{3+} by cysteinylmethionine (CysMe) (Scheme 1) [4]. In addition, vanadium accumulating ascidians dispose of an enhanced capacity to metabolize glucose-6 phosphate (G6P); the activity of G6P-dehydrogenase is markedly elevated when compared to ascidians with low accumulation rates [5]. The intermittently formed VO^{2+} binds to the lysine NH_2 residues of the vanabins. Vanabins are lysine-rich polypeptides of about 10 thousand kDa, attaining a bow-shaped conformation, with four α-helices connected by nine disulfide bonds [6-8].
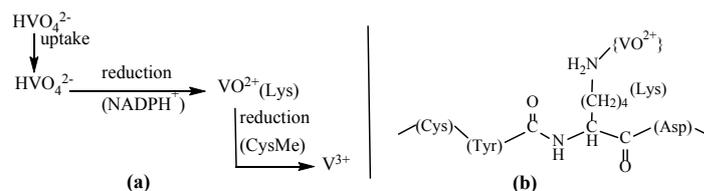
Scheme 1: **(a)** The step-wise reduction of vanadate (V) to vanadium (III). Cysteine-rich proteins (associated with the oligopeptide vanabin2) are involved in this process. **(b)** Section of vanabin2, illustrating the binding of VO^{2+} to a lysine residue.

The amount of vanadium accumulated by ascidians strongly differs. An extraordinary degree of vanadium – up to 350 mM and hence the about 10^7 fold of vanadate in sea water (~35 nM) – can be absorbed. In the intestinal lumen, the concentration can reach 0.7 mM. This particularly effective accumulation of vanadium, occurring in the greater part of the ascidians, apparently is supported by bacterial genera such as *Pseudomonas* and *Ralstoni* (in the tissues of the branchial sacs of the ascidians), and *Treponema* and *Borrelia* (in the intestines) [9-12] – a fact which is in line with the general ability of certain strains of bacteria from deep-sea hydrothermal vents (such as *Pseudomonas vanadium-reductans*) that reduce vanadate to vanadium in the +IV (and +III) state, using lactate as electron donor [13].

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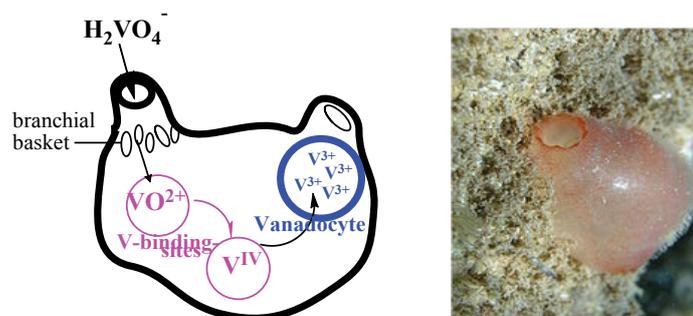
Figure 2: Schematic representation of the accumulation path of vanadium by ascidians (left) [8], exemplified here for *Ascidia gemmata* (right). Vanadate (V) enters the cell via phosphate/vanadate channels, supported by mutualistic bacteria. Vanadium binding sites are provided by lysine-rich polypeptides [6,9]. The blood cells storing V^{3+} are termed vanadocytes.

VANADIUM IN MARINE ALGAE

Several species of macro-algae in the marine environment (Figure 2) are able to catalyze the oxidation of the halide X^- ($X^- = I^-$, Br^-, Cl^-) to hypohalous acid [14-17], as exemplified for the bromide oxidation in eqn. (1). The oxidant employed by these algae is hydroperoxide (H_2O_2, HO_2^-); the oxidation is supported by heme-type or by vanadate-dependent enzymes, viz. haloperoxidases. Pseudohalides, such as cyanide and thiocyanate (eqn. (2)) can also be substrates. By producing hypohalous acid, the algae protect themselves against parasite infestation, in particular fungi. A few other groups of organisms also oxidize halides to hypohalite, among these marine *Streptomyces* [18], bacteria and cyanobacteria [19,20]. In the latter case, chlorinated compounds are formed that function as antibodies.

$$Br^- + H_2O_2 \rightarrow BrO^- + H_2O \quad (1a)$$
$$Br^- + H_2O_2 + H^+ \rightarrow HOBr + H_2O \quad (1b)$$
$$SCN^- + H_2O_2 \rightarrow OSCN^- + H_2O \quad (2)$$
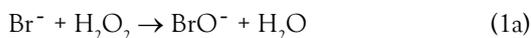
The production of volatile, highly oxidative hypohalous acids HOX by some macroalgae does also have implications for the climate, since – once released into the atmosphere – HOX is subjected to UV irradiation and forms radicals that contribute to the depletion of atmospheric ozone [21]; eqns. (3) and (4).

$$CH_3Br + h\nu \rightarrow Br + CH_3^{\bullet} \quad (3)$$
$$Br^{\bullet} + O_3 \rightarrow BrO + O_2 \quad (4)$$
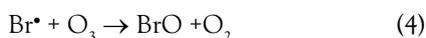
The active site (Figure 3) in all of these algae contains vanadium in an essentially trigonal-bipyramidal environment, in close hydrogen bonding interaction with surrounding water molecules and amino acid residues, cooperating with the active center via hydrogen bonds.

During turn-over, the VHPOs are self-supporting in the sense that they only marginally change their coordination environment. In the course of the reaction, a peroxido intermediate is formed, in which the trigonal-bipyramidal arrangement at the vanadium centre is essentially retained. H_2O_2 docks to the vanadium centre as hydroperoxide in a monodentate fashion, followed by the releases of one or two proton to form a η^2-peroxido and/or -hydro-peroxido intermediate. This intermediate undergoes a nucleophilic attacked by the substrate, such as bromide, and finally releases hypobromous acid. The several steps of this sequence are shown in Figure 4.
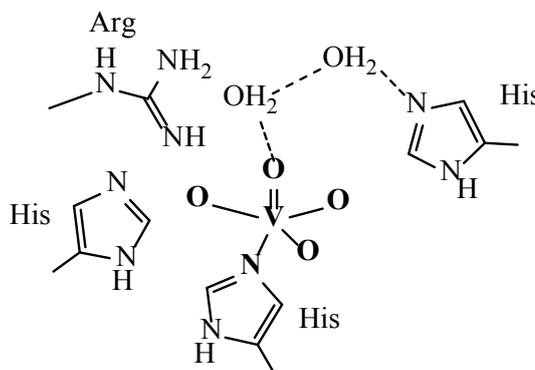
Figure 3: Section of the active centre of the bromoperoxidase from *Ascophyllum nodosum*, adapted and simplifies from ref [14].
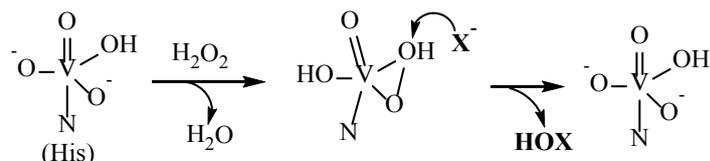
Figure 4: Activation of peroxide by the vanadium centre of the haloperoxidases (adapted, in part, from ref. [16]). The intermittently formed active hydroperoxido complex transfers a hydroxide to the halide X^-.

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