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Review

Vanadium accumulation in ascidians: A system overview

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ABSTRACT

Several families of ascidians accumulate extremely high levels of vanadium in their blood cells. The concentration of vanadium has been determined in each species; the highest concentration, found in *Ascidia gemmata*, reaches 350 mM, corresponding to 10^7 times that of sea water. How and why ascidians accumulate vanadium in a highly selective manner and at such extremely high levels have yet to be determined. To address these questions, our research group sought to identify the genes and proteins responsible for the accumulation and reduction of vanadium in vanadocytes, a type of blood cell, as well as the process of vanadium transport from sea water to blood cells through the branchial sac, intestine, and blood plasma. Here, we review the accumulation steps as a system, especially those related to the concentration and chemical species of vanadium at each step. A comprehensive analysis on each organ has already revealed several categories of protein families, such as vanadium-binding proteins and vanadium transporters. Herein, we also discuss the mechanisms by which ascidians selectively accumulate vanadium ions from a biochemical viewpoint.

1. Introduction

Several families of ascidians (sea squirts or tunicates) accumulate extremely high levels of vanadium in their blood cells. The concentration of vanadium has been determined in each species; the highest concentration, in *Ascidia gemmata*, was 350 mM, corresponding to 10^7 times that of sea water. How and why ascidians accumulate vanadium in a highly selective manner and at such extremely high levels have yet to be determined. To address these questions, our research group sought to identify the genes and proteins responsible for the accumulation and reduction of vanadium in vanadocytes, a type of blood cell, as well as the process of

Abbreviations: AAS, atomic absorption spectrometry; CW, constant-wave; DCT, divalent cation transporter; DTT, dithiothreitol; EPR, electron paramagnetic resonance; EST, expressed sequence tag; FISH, fluorescence *in situ* hybridization; GR, glutathione reductase; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; IMAC, immobilized metal ion affinity chromatography; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; Nramp, natural resistance-associated macrophage protein; OTU, operational taxonomic unit; V-ATPase, vacuolar H^+-ATPase.

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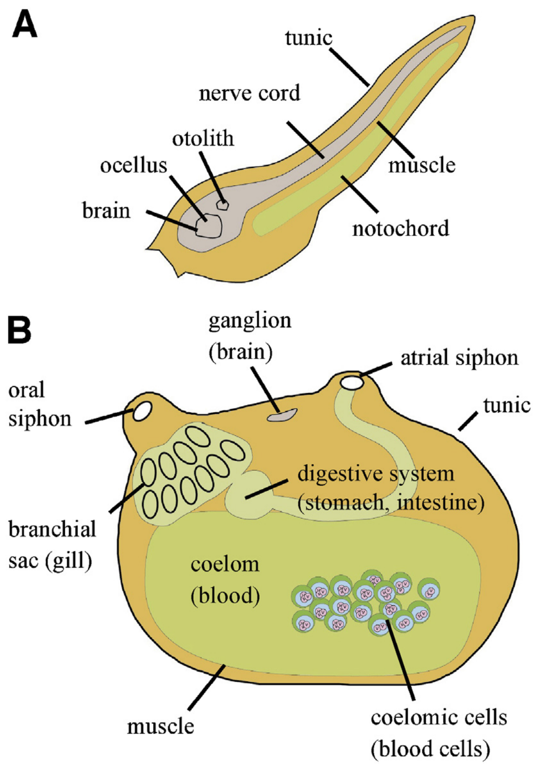

Fig. 1. Schematic illustration of the larval and adult forms of ascidians. (A) A typical larval form. The swimming larva possess head/trunk and tail regions, and can swim by the tail movement. Brain and sensory organs are located in head/trunk region. The larva attaches to a substrate, and metamorphose to the adult form by losing tails and rearranging the internal organs. (B) A typical adult form. It cannot locomote by itself. A single cerebral ganglion lies between two siphons.

vanadium transport from sea water to blood cells through the branchial sac, intestine, and blood plasma.

In this review article, we first briefly summarize previous studies on vanadium accumulation in ascidians. Next, we overview the accumulation steps as a system, especially those related to the concentration and chemical species of vanadium at each step. How an ascidian system takes up vanadium selectively is also discussed from biochemical properties of proteins responsible for each step. A more comprehensive review of the molecular mechanism for each step of accumulation was published in a previous issue of this journal [1].

2. Prior studies on vanadium accumulation in ascidians

Ascidians are marine sessile invertebrate animals belonging to Urochordata, Phylum Chordata. At the larval stage, the body plan is quite well-conserved with other chordates, but after metamorphosis, it is drastically reconstructed and takes on a unique shape (Fig. 1). Reflecting their phylogenetic position, they are good models for studying genomic evolution, early development, as well as the immune and nervous systems [2,3]. A cosmopolitan species, *Ciona intestinalis*, is an extremely good model; it was the seventh animal species whose whole genome was sequenced [4], and millions of expressed sequence tag (EST) data have been deposited in public databases [5–7]. In contrast, they possess unique features that are not found in other chordates, including cellulose synthesis [8–12], asexual reproduction [13,14], and metal accumulation [15–18].

Approximately 100 years ago, the German physiological chemist Dr. Martin Henze discovered high levels of vanadium in the blood (coelomic) cells of the ascidian *Phallusia mammillata* collected from the Bay of Naples, Italy [19]. His discovery attracted the interdisciplinary attention of chemists, physiologists, and biochemists, in part because of considerable interest in vanadium as a possible prosthetic group, in addition to iron and copper, in respiratory pigments. This would have implied a role for vanadium in oxygen transport, a hypothesis that later proved to be false [20].

Table 1
Concentration of vanadium, proton and sulfate in ascidians.

Ascidian species	Vanadium (mM)	pH	Sulfate (mM)	References
Ascidia gemmata[a]	347.2	1.86	500	[22,34]
Ascidia ceratodes[a]	99	1.8	250	[35]
Ascidia ahodori[a]	59.9	2.67	–	[22]
Ascidia sydneiensis samea[a]	12.8	4.20	210	[22,33]
Ascidia sydneiensis samea[b]	38	~5	86	[33]

[a] Values obtained from whole blood cells.
[b] Values obtained from blood cells with the exception of giant cells.

The concentration of vanadium within tissues of many ascidians has been determined by neutron activation analysis, EPR, or AAS. Ascidians belonging to the suborder Phlebobranchia contain higher levels of vanadium than those of the suborder Stolidobranchia [21] (Fig. 2). Of the tissues examined, blood cells contained the highest amount of vanadium. The greatest concentration was found in blood cells of the ascidian *A. gemmata*, at up to 350 mM [21,22], which is 10^7 times that in seawater (35 nM) [23,24]; this is believed to be the highest degree of accumulation of a metal in any living organism.

Ascidian blood cells can be classified into 9–11 types, mainly on the basis of their morphology [25]. Morula cells were long believed to be vanadium-containing cells, or vanadocytes. However, by direct measurement of vanadium in each blood cell type, it was determined that morula cells did not accumulate vanadium; rather, signet ring cells and some vacuolated cells are true vanadocytes [26,27]. A more detailed history is provided in previous reviews [1,15,28,29].

Dr. Henze, in addition to discovering very high levels of vanadium in blood cells, also reported that blood cell homogenate is extremely acidic and contains high levels of sulfur [19,30–32]. Reported pH values varied considerably in early studies. It is probable that one of the reasons for the variation in pH values is that the measurement was made without fractionation of the population of blood cells. Thus, an elegant study was designed to isolate acidic blood cells among several types of blood cell to determine whether acidic blood cells were identical to vanadocytes [22]. Microelectrode measurement of blood cell lysates and non-invasive EPR measurements on intact cells under anaerobic conditions revealed a correlation between the concentration of V^{III} ions and pH within the vacuoles of three *Ascidia* species [22,26]. Sulfate concentration was also measured by Raman spectroscopy in two *Ascidia* species [33,34]. Analytical and EPR spectroscopic measurements have been carried out in vanadocytes from *Ascidia ceratodes* [35] to reveal vanadium, proton and sulfate concentrations. Reported values are summarized in Table 1.

3. How do intestinal cells absorb vanadium?

Vanadium is a multivalent transition metal. Under physiological aqueous conditions, vanadium ions are limited to the +3, +4 and +5 oxidation states [36]. In nature, vanadium is usually in the +5 state (HVO_4^{2-} or $H_2VO_4^{-}$; V^V) [37], but the majority is reduced to the +3 state (V^{3+}; V^{III}) *via* vanadyl ions in the +4 state (VO^{2+}; V^{IV}) during assimilation and hyper-accumulation by ascidians [38–40]. Thus, the first questions to consider are how and where ascidians absorb vanadium as V^V anions.

The chemical features of V^V ions at low concentration resemble those of phosphate anions (HPO_4^{2-} or $H_2PO_4^{-}$; P^V) [16,41]. Fig. 3