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EDITORIAL

Recent perspectives into biochemistry of decavanadate

Manuel Aureliano

Manuel Aureliano, FCT, University of Algarve, Gambelas, 8005-139 Faro, Portugal
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Supported by Center for Marine Sciences funding
Correspondence to: Manuel Aureliano, PhD, Associate Professor, FCT, University of Algarve, Gambelas, 8005-139 Faro, Portugal. maalves@ualg.pt
Telephone: +351-289-800905 Fax: +351-289-800066
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Abstract

The number of papers about decavanadate has doubled in the past decade. In the present review, new insights into decavanadate biochemistry, cell biology, and antidiabetic and antitumor activities are described. Decameric vanadate species (V_{10}) clearly differs from monomeric vanadate (V_1), and affects differently calcium pumps, and structure and function of myosin and actin. Only decavanadate inhibits calcium accumulation by calcium pump ATPase, and strongly inhibits actomyosin ATPase activity (IC_{50} = 1.4 μmol/L, V_{10}), whereas no such effects are detected with V_1 up to 150 μmol/L; prevents actin polymerization (IC_{50} of 68 μmol/L, whereas no effects detected with up to 2 mmol/L V_1); and interacts with actin in a way that induces cysteine oxidation and vanadate reduction to vanadyl. Moreover, *in vivo* decavanadate toxicity studies have revealed that acute exposure to polyoxovanadate induces different changes in antioxidant enzymes and oxidative stress parameters, in comparison with vanadate. *In vitro* studies have clearly demonstrated that mitochondrial oxygen consumption is strongly affected by decavanadate (IC_{50}, 0.1 μmol/L); perhaps the most relevant biological effect. Finally, decavanadate (100 μmol/L) increases rat adipocyte glucose accumulation more potently than several vanadium complexes. Preliminary studies suggest that decavanadate does not have similar effects in human adipocytes. Although decavanadate can be a useful biochemical tool, further studies must be carried out before it can be confirmed that decavanadate and its complexes can be used as anticancer or antidiabetic agents.

Key words: Decavanadate; Vanadate; Calcium pump; Myosin; Actin; Actin polymerization; Insulin mimetic; Antidiabetic agent; Antitumor agent

Peer reviewers: Emanuel E Strehler, PhD, Professor, Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905, United States; Beth S Lee, PhD, Associate Professor, Physiology and Cell Biology, The Ohio State University, 1645 Neil Avenue, 304 Hamilton Hall, Columbus, OH 43017, United States

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INTRODUCTION

The number of articles about vanadium in the past decade (2001-2010) has doubled in comparison to the previous one (1991-2000), from 1149 to 2616, of which, 74 (48 in the previous decade) are about decavanadate. A relevant contribution towards our understanding of the effects of vanadium in the environment, biochemistry, biology and health was published in 1998[1]. In the past decade, at least five reviews on different aspects of vanadium have been published, covering chemistry, chemical engineering, biochemistry, biology, pharmacology and medicine[2-6], which is a testimony to the recent interest in this transitional metal in several scientific areas. However, there have been few studies about decavanadate and only seven *in vivo* studies have been published in the past decade[7-13]. These *in vivo* studies have demon-

strated that decavanadate in animals induces different changes in vanadium accumulation, lipid peroxidation and antioxidant enzyme activity than those observed for monomeric vanadate, and consequently it can also contribute to the effects described for vanadium. Therefore, the different changes in oxidative stress markers and lipid peroxidation, among others, can be attributed to decavanadate[7-13]. In several kinetic studies, following decavanadate administration, nuclear magnetic resonance (NMR) and UV/Vis spectroscopy have been used to correlate the vanadate species with the observed biological effects[7-13].

Decavanadate is well known to interact with several proteins and to have many biological activities, mainly *in vitro,* as recently reviewed[14,15]. The first enzyme reported to be inhibited by decavanadate was muscle adenylate kinase[16]. Other enzymes included hexokinase, phosphofructokinase and inositol phosphate metabolism enzymes[17,18]. In the past decade, it has been demonstrated in our laboratory that decavanadate interacts with calcium ATPase, myosin and actin, suggesting that it can affect several biological processes, such as muscle contraction and its regulation, actin polymerization, and calcium homeostasis[19-23]. We believe that in many studies using vanadate, decavanadate species will form, and therefore they will contribute eventually to the described biological effects[14,15]. Decavanadate can be more or less effective than the corresponding simple oxovanadates[14-18].

Since it was discovered that ATP from Sigma contained vanadium[24], vanadium has been used as a tool to understand several biochemical processes[14,15]. Moreover, vanadate, is actually accepted as a potent inhibitor of protein tyrosine phosphatase (PTP), a key enzyme in the insulin signaling pathway. PTP is described as one the main targets of vanadate as an insulin mimicking agent, promoting an increase in glucose uptake in several types of cells[25].

In the present review, we describe recent insights into the effects of decavanadate on muscle proteins, such as myosin, actin and calcium pump, as well as its toxicological effects *in vivo* and, more recently, its antidiabetic and anticancer effects. Some comparisons will be made with the vanadyl cation, the tetravalent form of vanadium, which, although in the majority intracellularly, is not the main focus of this review, and the reader is referred elsewhere[26,27]. Although we present new data about the interaction of vanadate, decavanadate and vanadyl with actin, the main purpose is to highlight recent insights into decavanadate biochemistry, which are not usually taken in account in biological studies of vanadium.

COMPLEX CHEMISTRY OF VANADIUM: CAN WE BE CERTAIN ABOUT WHICH VANADIUM SPECIES ARE INDUCING THE BIOLOGICAL EFFECTS?

Can the complex chemistry of vanadium explain the diversity of its biological effects? Even in 2011, many studies using vanadium clearly misunderstand its chemistry, which leads to wrong conclusions and research directions in an attempt to clarify the biological role of vanadium. The complexity of vanadium chemistry in aqueous solutions includes: (1) several oxidation states; (2) chemical similarity of vanadate and vanadium (V) with phosphate; (3) ability to form vanadate oligomers, such as decavanadate; (4) capacity to form complexes with many molecules of biological interest such as ATP, ribose, glutathione and amino acids, through different coordinating atoms such as oxygen, nitrogen and sulfur; (5) ability of having several geometric configurations, some mimicking enzyme substrate transition state analogs; (6) low solubility in aqueous solutions of some vanadium complexes of biological interest; and (7) low stability of many vanadium complexes used as insulin mimetics or anticancer agents under physiological conditions and at 37 ℃[3,5,6,14,15,18,28].

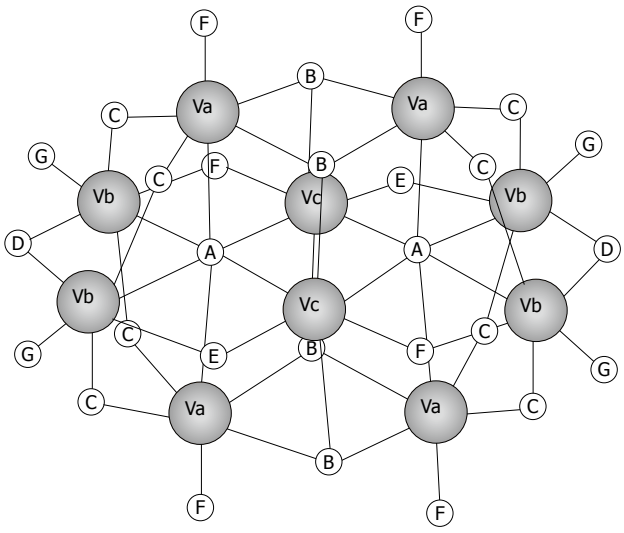

Figure 1 Schematic structure of V_{10} ($V_{10}O_{28}^{6-}$). Va, Vb and Vc represent the three different types of vanadium atoms described in the text.

Vanadate [vanadium (V)] generates a variety of different oxovanadates, depending on pH, concentration and specific conditions[26,29]. The simple vanadate colorless solution contains several metavanadate species (VO_3^-), depending on vanadium concentration, such as monomeric vanadate [V_1, orthovanadate species (VO_4^{3-})] dimeric vanadate (V_2), tetrameric vanadate (V_4) and pentameric vanadate (V_5)[15]. If, after preparation of a stock vanadate solution (for instance 10 mmol/L), acidification occurs, the instantaneous appearance of a yellow color indicates the formation of decavanadate, even if the overall pH value of the solution is not acidic, that is, does not change significantly[14,15]. Therefore, when using vanadate solutions in chemical, biochemical or biological studies, even at physiological pH values, it is critical to avoid acidification steps, unless decavanadate species are desirable[14,15]. Decavanadate, with a formula of $V_{10}O_{28}^{6-}$[15,30], has a unique structure, with dimensions of 8.3 Å, 7.7 Å and 5.4 Å (Figure 1). By ^{51}V NMR spectroscopy, three

different types of vanadium atoms can be distinguished (Va, Vb and Vc; Figure 1), whereas by UV/Vis spectroscopy, specific absorption at 360 and 400 nm, attributed to decavanadate species, can be detected, which is responsible for the typical yellow or bright orange of vanadium solutions[14,15,30]. In spite of this knowledge, many studies still misinterpret the chemistry of vanadium in solution particularly, and do not recognize that, if the vanadate solution turns yellow, this is due to the formation of decameric vanadate species[14,15]. Similarly, if the solution turns blue, this means that all the decameric vanadate has decomposed to the monomeric form of vanadate (colorless), followed by vanadium reduction to the vanadyl species that confers the blue color on the solution. Eventually, the observation of a green color during this process is due to the mixture of vanadyl (blue) and decavanadate (yellow) species.

Once formed even at neutral pH, decavanadate can be removed by two procedures; the most convenient method is to heat or boil the solution[31]. Alternatively, the solution can be aged. Depending on the pH, the decavanadate will ultimately convert to the colorless metavanadates or orthovanadates. As described above, it is important to recognize that generally a yellow color of these vanadate solutions reflects the fact that some decavanadate is present in the solution, and should be removed by heating if this species is not desired[31,32]. ^{51}V NMR spectroscopy can be used to monitor the speciation of oxovanadates in biological systems and experiments can be designed to evaluate specific interactions of the different vanadate oligomers with compounds in the biological system[33-35]. Decavanadate stability can be followed by UV/Vis spectroscopy, even for μmol/L concentrations, due to absorption in the ultraviolet region that confers the yellow color observed for decavanadate solutions[35,36].

Conversely, the lack of stability of some vanadium complexes used as antidiabetic or anticancer agents can contribute to misinterpretation about the role of vanadium in biology, namely its putative application as a therapeutic agent. In fact, in the majority of studies published describing the effects of vanadium complexes on biological systems, the authors have not taken into account the stability of vanadium complexes, which are often incubated with cells during long periods of time, and particularly at 37 ℃, which decreases vanadium complex stability. In fact, even vanadium complexes such as bis-maltolato-oxovanadium (IV) (BMOV), which is known for its insulin mimetic effects, decompose and are oxidized, even at 25 ℃[37]. By combining several spectroscopic techniques, it is possible to analyze the stability of the vanadium compounds and to confirm which species are truly present in the medium at the time they are promoting the observed effects, and even after inducing the effects. Therefore, without a clear demonstration that the vanadium species are present in the medium, and that the vanadium complexes or species have not decomposed, it can be only speculated that the observed effects are due to the vanadium compound that has been added to the medium.

DECAVANADATE INTERACTIONS WITH CALCIUM PUMP FROM SARCOPLASMIC RETICULUM

Sarco/endoplasmic reticulum calcium ATPase, a member of the E1E2 or P-type ATPase family, is present in two main conformations, E1 and E2, during the process of calcium translocation. It has been established that the E1 state is prevalent in the presence of $Ca^{2+}$and the E2 state in the absence of Ca^{2+}[38]. Moreover, E1 can be phosphorylated by ATP but not by inorganic phosphate, whereas E2 can be phosphorylated by inorganic phosphate but not by ATP. The catalytic site of sarcoplasmic reticulum (SR) Ca^{2+}-ATPase contains an aspartyl residue that is phosphorylated by ATP during the catalytic cycle, forming an acyl phosphate anhydride[38]. In the E1 conformation, the protein captures Ca^{2+} from the cytoplasm and is phosphorylated by ATP to form E1-P(Ca), which then changes its conformation to E2-P with a concomitant loss of affinity for the Ca^{2+}, releasing it into the lumen. Subsequently, the enzyme phosphorylated in the conformation E2 suffers hydrolysis, then E2 turns into E1, and the cycle is again initiated[38,39].

Vanadate is well known as a specific inhibitor of the SR Ca^{2+}-ATPase[40-43]. Several kinetic studies have suggested that vanadate inhibits SR-ATPase by forming a transition state analog of the phosphorylated intermediate, blocking the E2 conformation of the protein[40]. Decavanadate also interacts with the SR calcium pump[41], at a distinct site from the phosphorylation site. Decavanadate can also interact with other protein conformations such as E1, E1-P and E2-P, contrary to monomeric vanadate, as described by ^{51}V-NMR spectroscopy[35,42]. Only decavanadate, and not vanadate, is able to inhibit calcium accumulation coupled with ATP hydrolysis in SR vesicles, as well as proton ejection by the (SR) Ca^{2+}-ATPase[35,42,43].

SR calcium pump has proven to be an excellent model to study toxicology effects of oxovanadates and vanadium complexes on E1E2-ATPases, such as the E1E2-Na^+, K^+-ATPase and Ca^{2+}-ATPase, once they are involved in essential ion homeostasis, such as Ca^{2+} homeostasis, therefore regulating several processes in muscle and non-muscle cells. The calcium pump from SR has previously been shown to address metals toxicity, once it was found to be inhibited by oxovanadates, such as decavanadate and tetrameric vanadate, vanadium citrate complexes and BMOV, among others[37,42-44]. Moreover, several conditions of calcium accumulation coupled or not coupled with ATP hydrolysis, can be addressed using vesicles from SR calcium ATPase and the effects of several oxovanadatates evaluated (Figure 2). The measurements of Ca^{2+} accumulation by the SR calcium pump can be performed when the calcium uptake is coupled with

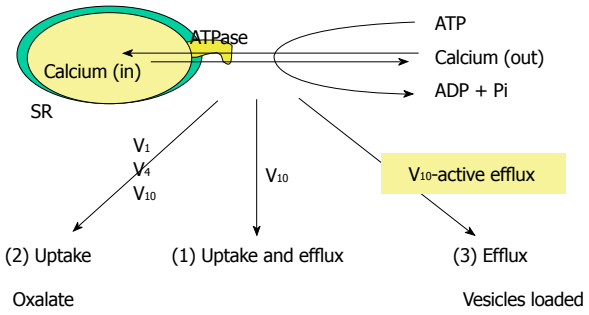

Figure 2 Modes of calcium translocation by SR calcium pump as affected by different vanadate oligomers. V_1: Monomeric vanadate; V_4: Tetrameric vanadate; V_{10}: Decameric vanadate. Only V_{10}, and not V_1, was shown to inhibit calcium uptake in conditions 1 and 3, that is, when ATPase activity is coupled to calcium transport. V_1 only inhibits the ATPase in condition 2, where the calcium gradient is destroyed by oxalate or phosphate.

ATP hydrolysis, therefore mimicking physiological conditions (Figure 2, condition 1, coupled uptake). At this condition, where a gradient of calcium modulated the calcium pump activity, only decameric vanadate (V_{10}) inhibits the calcium pump (Figure 2). In another condition, after filling the vesicles with calcium, when the efflux of calcium is coupled to ATP synthesis, it is observed that only decavanadate inhibits this process, whereas mmol/L concentrations of V_1 have no effect (Figure 2, condition 3, active efflux). In another different experimental condition, it is observed that, when the gradient of calcium is destroyed, meaning using phosphate or oxalate to reduce the calcium concentration inside the vesicles to almost zero, and the ATPase activity is at a maximum and we see mainly calcium uptake, calcium ATPase is inhibited by both V_{10} and V_1 solutions (Figure 2, condition 2, uncoupled calcium uptake)[19,32,35,43].

DECAVANADATE INTERACTIONS WITH SKELETAL MUSCLE MYOSIN

The mechanism of myosin ATPase inhibition by the monomeric vanadate species has been relatively well characterized; little has been reported about the inhibition of the process of muscle contraction by decavanadate. Whereas, monomeric vanadate (HVO_4^{2-}), mimics the transition state for γ-phosphate hydrolysis, at the active site[44], blocking myosin in a power-stroke state, by mimicking the ADP.Pi intermediate state, the decavanadate mode of action implies a binding site different from the ATP binding site. In fact, recent kinetic studies have shown that, unlike vanadate, decameric species were able to inhibit strongly the myosin or myosin subfragment-1 (S1) actin-stimulated ATPase activity with an IC_{50} of 6.11 ± 0.74 and 1.36 ± 0.14 μmol/L V_{10} for myosin and S1 (myosin subfragment S1, respectively, whereas no inhibitory effects were detected for vanadate up to 150 μmol/L[5,15,20]. A detailed kinetic analysis, revealed that decavanadate inhibition is non-competitive, yielding an inhibition constant Ki = 0.27 ± 0.05 mmol/L[20].

Another feature that distinguished the inhibition of the actomyosin complex by vanadate and decavanadate is the interaction of actin with myosin. Myosin-ADP-V_1 complex is destabilized by F-actin, inducing the release of the products, whereas myosin-MgATP-V_{10} is not. Therefore, only decavanadate prevents the release of the products during ATP hydrolysis by the actomyosin complex[20,21], inhibiting the stimulation of the myosin ATPase activity by actin. Apparently, decavanadate ($V_{10}O_{28}^{6-}$), induces the formation of the intermediate myosin-MgATP-V_{10} complex blocking the contractile cycle, most probably in the pre-hydrolysis state[20,21]. Although many aspects of the interaction of decavanadate with the process of ATP hydrolysis by the actomyosin complex is not completely understood, we infer that different oxovanadates are able to populate different conformational states of the myosin ATPase cycle depending on their oligomerization state. It is proposed that decavanadate inhibits myosin ATP hydrolysis, as well as F-actin stimulation of the release of the products, blocking ATP hydrolysis by the actomyosin complex, probably in the pre-hydrolysis state or before the interaction between actin and myosin, as shown schematically (Figure 3).

The walker A motif (corresponding to the P-loop in myosin) of ATP-binding cassette ATPases, is an anion-binding domain that can bind decavanadate with high affinity[45]. With myosin, decavanadate interacts with the phosphate-binding domains, in the vicinity of the nominated "back-door" binding site, interfering with movements associated with ATP hydrolysis by the actomyosin complex, therefore, by forming the intermediate myosin-MgATP-decavanadate complex[5,15,20,22]. The interaction of myosin with vanadate and decavanadate have mainly been described *in vitro* using skeletal muscle myosin (type Ⅱ myosin), whereas studies with non-muscle myosins, with cells or muscle fibers, using decavanadate have been scarce or non-existent. Some studies have reported the effects of vanadate in muscle fibers[46,47].

DECAVANADATE INTERACTIONS WITH SKELETAL MUSCLE ACTIN

To the best of our knowledge, before the past decade, only three studies were performed to investigate the interaction of vanadium with actin[48-50]. Vanadate has been shown to increase actin-actin interactions similarly to phosphate[48], and it also induces distinct effects on actin polymerization rather than phosphate[49]. Another study has analyzed vanadyl [vanadium (IV)] interaction with the monomeric actin, G-actin, revealing the presence of one strong vanadium binding site[50,51].

Contrary to myosin, not much information is available at the molecular level about decavanadate interaction with actin. The first study to describe the interaction between decavanadate and actin has suggested that actin, under certain experimental conditions, stabilizes the decomposition of decavanadate by increasing the half-life from 5 to 27 h, whereas no effects are detected upon myosin[36]. Moreover, it has been reported that decavana-

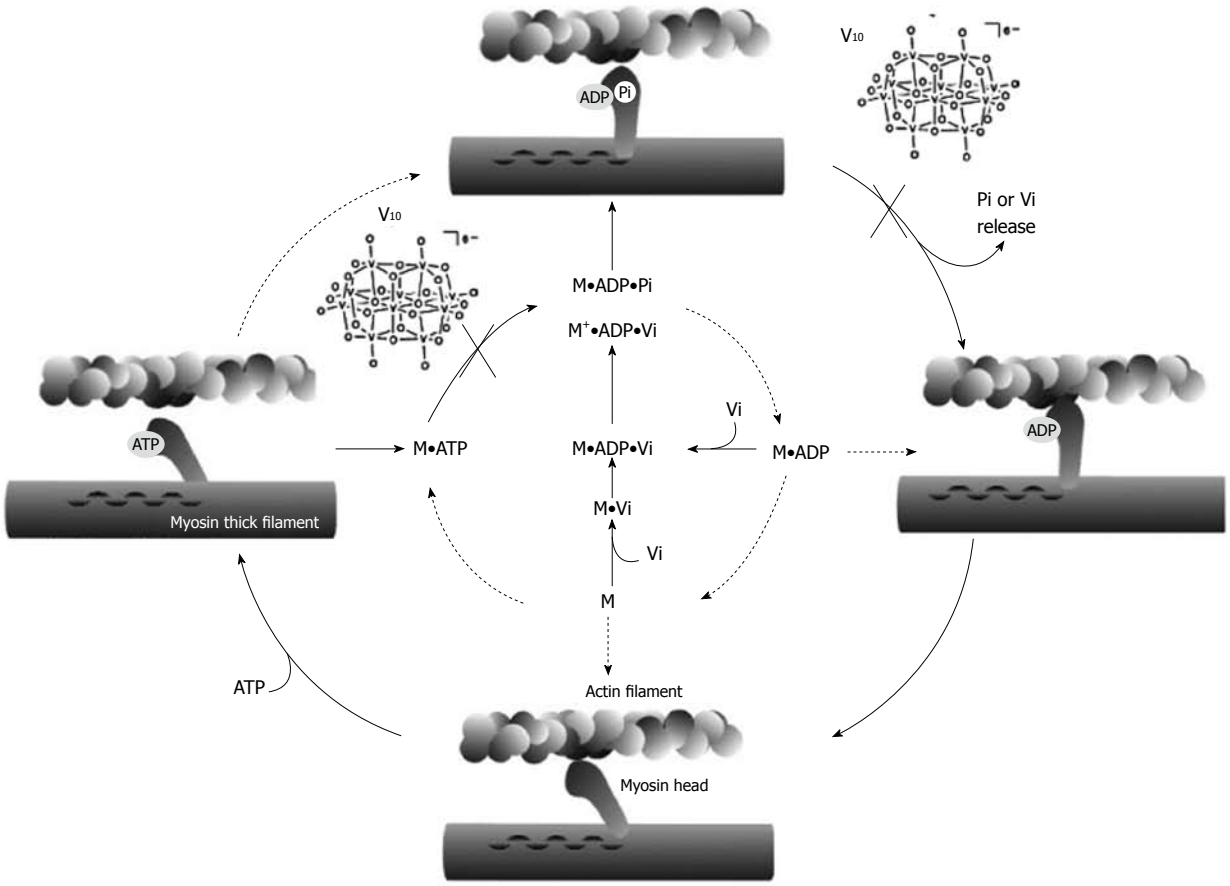

Figure 3 Relevant steps of ATP hydrolysis by actomyosin complex. The dominant process of ATP hydrolysis observed *in vitro* is indicated by the filled arrows. M: Myosin; Vi: Orthovanadate; V_{10}: Decavanadate. V_{10}, can blocked the process at two steps, without or with F-actin: before ATP hydrolysis and before product release, just before power stroke.

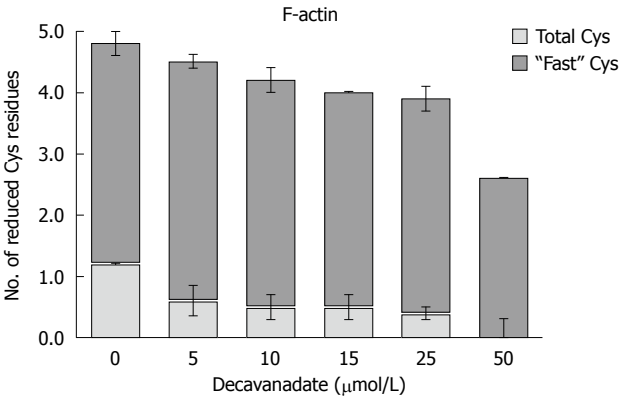

Figure 4 F-actin cysteine redox state, after 20 min exposure to decavanadate. Titration of cysteine was performed with 0.1 mmol/L 5,5'-ditiobis-(2-nitrobenzoic acid) and 2 μmol/L actin in 2 mmol/L Tris (pH 7.5), and 0.2 mmol/L $CaCl_2$. The increase in absorbance at 412 nm was continuously recorded over 10 min; To measure total cysteines the samples were treated afterwards with 1% SDS, and the absorbance was measured, over 15-30 min, until a steady value was reached. Titration with decavanadate produced a dose-dependent decrease in F-actin total cysteines, while Cys-374 (also named "fast cysteine") is reduced. The results shown are the average of triplicate experiments.

date inhibits the rate of G-actin (monomeric form of actin) polymerization into F-actin (polymerized form of actin), with an IC_{50} of 17 μmol/L[36], suggesting that it affects cytoskeleton structures responsible for many biologically significant processes. It has recently been reported that the interactions of decavanadate with actin induce protein cysteine oxidation and vanadate reduction[23,52].

It has been observed that only V_{10} solution, but not vanadate, is able to oxidize F-actin Cys-374 (also named "fast Cys") and one of the protein core cysteine residues (Figure 4), whereas for G-actin, only the latter effect is observed[52]. As described using NMR spectroscopy, ATP protects the actin from interaction with decavanadate, and prevents cysteine oxidation[23,52]. It has been demonstrated that decavanadate interactions with actin are of particular interest once it was observed that only V_{10} species are able to promote protein cysteine oxidation. However, does actin cysteine oxidation imply decavanadate reduction to vanadyl? In fact, decavanadate interaction with both G- and F-actin results in concomitant reduction of vanadate to vanadyl [vanadium at oxidation state (IV)][23,52]. Typical EPR vanadium (IV) signals can be detected upon decavanadate incubation with actin, whereas the presence of ATP in the medium once again prevents decavanadate reduction to vanadyl, as recently described[23,52]. EPR titration of vanadyl with G-actin shows that vanadyl binds to actin with a K_d of

Table 1 Decavanadate *in vivo* studies in 2001-2010

Tissue	Effects	Administration mode	Exposition time	Ref.
H	Antioxidant enzymes	ip	1, 7 d	[7]
H/K/L	Histological effects	ip	1, 7 d	[8]
L	Vanadium accumulation Antioxidant enzymes	iv	12, 24 h, 7 d	[9]
H/B	Vanadium accumulation	iv	1, 6, 12 h	[10]
H	Lipid peroxidation Antioxidant enzymes	iv	1, 6, 12 h	[11]
H	Vanadium accumulation Antioxidant enzymes	iv	1, 7 d	[12][1]

[1]Comparison between vanadium (decavanadate, vanadate) and cadmium (5 mmol/L) administration. H: Heart; K: Kidney; L: Liver; B: Blood.

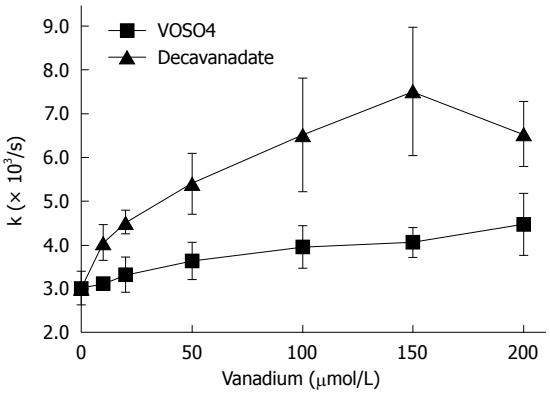

Figure 5 Exchange of bound ε-ATP of G-actin with ATP. Actin monomers (5 μmol/L) were incubated for 20 min with 0-200 μmol/L decavanadate, in 2 mmol/L Tris-HCl (pH 7.5), 0.2 mmol/L $CaCl_2$. The nucleotide exchange was monitored by the fluorescence decrease (λex = 350 nm; λem = 410 nm), as ε-ATP was replaced by ATP. Data are plotted as mean ± SD. The results shown are the average of triplicate experiments.

7.48 ± 1.11 μmol/L for G-actin and 43.05 ± 5.34 μmol/L for F-actin, with stoichiometry of approximately one and four vanadyl (VO^{2+}) cations bound per G- or F-actin molecule, respectively[52]. As described above for decavanadate, ATP prevents the interaction between vanadyl and actin, and therefore the observation of vanadyl EPR signals[52]. Both vanadyl and decavanadate interact with actin, but it has been shown that they induce different effects on protein structure and function, such as on myosin ATPase activity stimulated by F-actin, and actin polymerization, whereas decavanadate induces more potent effects on these two processes[23,52,53]. The effects of both vanadium species on actin structure have been compared, such as in protein intrinsic fluorescence, ATP exchange rate, and protein hydrophobicity[23,52,53]. It has been observed that decavanadate induces a more pronounced effect on the rate of ATP exchange rate, denoting a more open active site binding cleft (Figure 5). Decavanadate and vanadyl (up to 200 μmol/L total vanadium) both increased ε-ATP exchange rate ($k = 6.5 \times 10^{-3}$/s and 4.47×10^{-3}/s, respectively, in comparison with the controls: $k = 3.0 \times 10^{-3}$/s)[23,50,51], which clearly supports structural alterations to the actin ATP binding site.

TOXICOLOGY OF DECAVANADATE: *IN VIVO* AND *IN VITRO* STUDIES

In vivo studies of decavanadate administration have been performed since 1999, to understand the contribution of decameric vanadate species to vanadate toxicological effects[7-13]. Several experimental conditions have been used: (1) mode of decavanadate administration (intraperitoneal and intravenous); (2) fish species [*Halobatrachus didactylus* (*H. didactylus*)- Lusitanian toadfish - and *Sparus aurata* - gilthead seabream]; (3) vanadate concentration (1 and 5 mmol/L); (4) tissues (cardiac, hepatic, renal and blood); (5) subcellular fractions (cytosol, mitochondria, red blood cells and blood plasma); and (6) exposure time (1, 6, 12 and 24 h, and 2 and 7 d) (Table 1). A vanadate solution, not containing decameric vanadate species, was always administered for comparison. Following *in vivo* administration of decavanadate and vanadate solutions, several parameters were analyzed, such us: (1) vanadium subcellular distribution[7,9-11]; (2) histological changes in cardiac, hepatic and renal tissues[8]; (3) effects on SR Ca^{2+}-pump[13]; (4) lipid peroxidation; and (5) antioxidants enzyme activity and several oxidative stress markers in the heart[7,9,11] and liver[9]. It has been demonstrated that antioxidant stress markers, lipid peroxidation and vanadium subcellular distribution are dependent on the nature of the oxovanadates present in the administration fluid[7-13]. These studies have shown that, upon decavanadate administration, many effects are found that are not observed with vanadate, and conversely, many effects of vanadate are not observed with decavanadate.

Among several differences described, superoxide anion radical ($O_2^{\cdot-}$) production in mitochondria shows a dramatic difference upon decavanadate administration in comparison to vanadate[12]. $O_2^{\cdot-}$ production decreased by 35% in decavanadate-treated fish, whereas vanadate administration increased the $O_2^{\cdot-}$ production by 45%[12]. It should be noted that fish are very good models and adequate for these studies, because the physiological animal temperature (20-22 ℃) prevents decavanadate decomposition, and therefore, the effects can be seen. Therefore, pronounced increase of reactive oxygen species (ROS)

occurs in cardiac mitochondria following intravenous vanadate exposure, whereas decavanadate administration seems to prevent this effect. In *H. didactylus*, decavanadate (5 mmol/L, intraperitoneal) also induces a decrease in cardiac mitochondrial catalase activity (-60%) after 7 d. Taken together, these studies demonstrate that decavanadate exerts marked *in vivo* effects, with reactivity different from that obtained with simple vanadate.

Some of the above *in vivo* studies have demonstrated that following decavanadate administration, the mitochondrial fraction tends to accumulate more vanadium[7,11]. Moreover, decavanadate has specific effects on mitochondrial antioxidant enzyme activities[10,11]. Once again, these results confirmed that decavanadate behaves differently from vanadate. However, further studies will be required to clarify the importance of decavanadate for the biological effects of vanadium. We hope that others research groups will follow this direction.

During the studies described above, it has been observed that mitochondria accumulate vanadium, particularly when decavanadate is administrated. To explore further this association between mitochondria and decavanadate, studies were performed *in vitro*. In both hepatic and cardiac mitochondria, decavanadate inhibits mitochondrial respiration and induces mitochondrial membrane depolarization to a larger extent than monomeric vanadate[54]. For instance, decavanadate concentration as low as 100 nmol/L, inhibits 50% of oxygen consumption in mitochondria, while a 100-fold higher concentration of V_1 (10 μmol/L) is needed to induce the same effect. Moreover, decavanadate also induces mitochondrial depolarization (IC_{50} = 0.5 μmol/L) much more strongly than vanadate (IC_{50} = 50 μmol/L). These studies support the possibility that mitochondria are a potential cellular target for decavanadate[11,54,55]. Besides these mitochondrial effects of decavanadate (Table 2), in the past decade, several studies have shown that decavanadate has specific targets and many biological activities[5,14,15].

It was previously suggested that decameric vanadate species may eventually occur intracellularly in the cytosol, which is not acidic, upon acidification promoted by a chemical reaction or by an ionic pump[56]. However, based on vanadate chemistry, interconversion can occur in acidic compartments such as endosomes and lysosomes[14]. Therefore, the compartmentalization of different pH-containing domains in the cell favors the formation of decameric vanadate species[14]. After formation, decavanadate binds to specific protein binding sites, thus inducing different cellular responses from those of the other vanadate species (Figure 6). Therefore, a role of decameric vanadate species in biological chemistry is suggested[5,14,15].

Another potentially interesting feature of the effects of vanadate and decavanadate within cells is while the cytosol is at neutral pH, the membrane-bound intracellular compartments of the endocytic and secretory pathways are acidic. Therefore, the mode of entry into the cell plays a role in whether decavanadate is formed from monomeric vanadate (V_1). This further suggests that different cellular compartments might be differentially exposed to decavanadate. Although, the compartmentalization of vanadate species in cells is a subject still to be clarified, it has been proposed that V_{10} can be formed in acidic compartments in cells treated with vanadate, and ultimately is extruded into the medium. This confirms the possibility that V_{10} forms intracellularly[14]. Once outside the cells, decavanadate can cross membranes through specific anionic channels (Figure 6).

RECENT INSIGHTS INTO DECAVANADATE BIOLOGICAL AND BIOMEDICAL APPLICATIONS: INSULIN MIMETIC AND ANTITUMOR AGENT

Although many researchers remain skeptical whether decavanadate has a physiological role, in the past decade, several contributions have demonstrated that decavanadate induces relevant biological activities, which may eventually have a relevant impact in medicine (Table 2). By 2011, several studies about new decavanadate complexes, as well other polyoxometalates, have been published, and the potential medical applications are increasing, namely as insulin mimetic agents, inhibitors of aggregation of amyloid β-peptides associated with Alzheimer's disease, and as antitumor agents[5,14,15,57-61].

It is estimated that, by 2025, about 300 million people will have diabetes mellitus. Diabetic patients are also subject to other pathologies such as nephropathy, and arterial and neurodegenerative diseases. Vanadium, is well known to have insulin like or insulin-enhancing effects in several animal model systems[29,62-64]. These effects are probably induced through the inhibition of PTPs, as described above. However, vanadium may also, eventually, take action through ROS generation, and it is well known that transitional elements, such as vanadium, promote Fenton-like reactions. These actions could explain, at least in part, the antitumor effects of vanadium[65]. As an antidiabetic agent, vanadium has been described to act through an insulin-dependent or -independent pathway[29,62-66], although the mechanisms of action are still to be clarified.

Select polyoxometalates have been found to have insulin-enhancing properties[67], and recently, we have reported that the effect of decavanadate on glucose uptake in rat adipocytes was sixfold greater than the control level, and was more effective than BMOV and other vanadium complexes[68]. However, preliminary studies in human adipocytes (unpublished data) have shown that the effects described in rat adipocytes cannot be extrapolated to humans, after no similar effects were detected on glucose accumulation[69]. Several studies using decavanadate complexes have promoted the use of polyoxometalates as a tool for the understanding of many biological processes, including as antidiabetic and antitumor agents[5,14,15,57-60]. Medical applications of vanadium have been promoted in studies focusing on the structure-activity relationship

Table 2 Decavanadate *in vitro* studies in 2001-2010

Protein/effect	Vanadate species	Yr	Ref.
DNA-binding protein	V_{10}	2002	[70]
Methemoglobin reductase inhibition	V_{10}	2003	[73]
Actomyosin ATPase inhibition	V_{10}	2004	[20]
Muscle contraction regulation	V_{10}	2004	[21]
ATP sensitive cation -channel	V_{10}	2004	[76]
TRPM4 cation channels	V_{10}	2004	[71]
G-Actin polymerization inhibitor	V_{10}, V_4	2006	[36]
RNA triphosphatase	V_{10}	2006	[72]
P2X receptor antagonist	V_{10}	2006	[74]
Insulin mimetics	V_{10} compounds	2007	[57]
Back-door binding to myosin	V_{10}	2007	[22]
Porin (VDC) modulator	V_{10}	2007	[75]
Mitochondrial membrane depolarization	V_{10}, V_1	2007	[54]
Mitochondrial oxygen consumption	V_{10}, V_1	2007	[55]
Extracellular matrix mineralization	V_{10}, V_1	2008	[77]
Cardiomyocytes necrotic cell death	V_{10}, V_1	2008	[78]
Gelatine-mixtures	V_{10}	2008	[79]
Adipocytes glucose accumulation	V_{10}	2009	[68]
Actin oxidation and vanadyl formation	V_{10}	2009	[52]
Anticancer activity	V_{10} compounds	2009	[58]
ATPase activity in synaptic membranes	V_{10}	2009	[80]
Membrane models interaction	V_{10}	2009	[81]
DNA cleavage	V_{10}	2010	[82]
Actin structure and function	V_{10}	2010	[2]
Anticancer activity	V_{10} compounds	2010	[59]

TRPM4: Transient receptor potential cation channel subfamily M member 4.

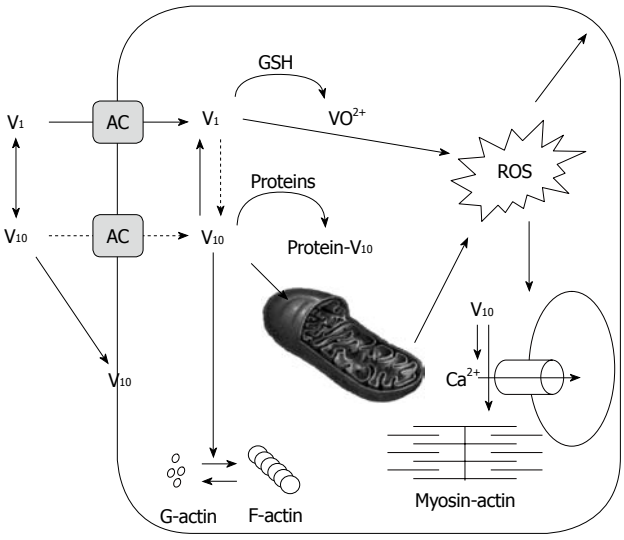

Figure 6 Scheme of proposed decavanadate (V_{10}) cellular targets. V_{10} uptake through anionic channels (AC). Decavanadate might interact with membrane proteins. V_{10} formation upon intracellular vanadium acidification in cytosol, but most probably in acidic organelles. Reduction of monomeric vanadate (V_1) by antioxidant agents. Binding of V_{10} to target proteins; it is proposed that V_{10} accumulates in subcellular organelles, such as mitochondria, affecting its function. Decavanadate also targets the contractile system, and its regulation, as well as calcium homeostasis (adapted from[14]).

of antidiabetic vanadium complexes, and vanadium compounds as antitumor drugs, to make vanadium available and safe for clinical use.

As described above, the *in vivo* studies of decavanadate administration in fish models took into account that decavanadate is sufficiently stable, and therefore the biological effects can be revealed. In studies with other animal models, and at different physiological temperatures, decavanadate stability will be different. In fact, it has been verified *in vivo* that decavanadate, at room temperature, has a half-life in serum of 15 h[7]. Moreover, in kinetic *in vitro* studies, performed at 25 ℃ or 37 ℃ (mitochondria studies) the half-life was 12 and 3 h, respectively[7-9,78]. The kinetic studies were always performed using a reaction time much less than the stability of decavanadate, between 10 to 30 min, to ensure that the biological effects were mainly due to decavanadate[7-13,52]. Therefore, the stability of the vanadate species is a very important factor in the biological effects of decavanadate. However, as described above, in the majority of the vanadium studies, the stability of the vanadate species or the vanadium complexes was not taken in account. In these studies, we can only speculate that the observed effects might have been due to the vanadium compound. Even for vanadium complexes, which are known to induce several insulin mimetic effects, it was verified that other species can be formed, even with other vanadium oxidation states than the original one[37]. Therefore, besides the factors described for vanadium complex chemistry, we may add the importance of certifying the stability of the vanadium complexes or species before attempting to attribute to them a certain biological activity or effect.

CONCLUSION

These studies have revealed the biological chemistry of decavanadate; a vanadate oligomer that eventually occurs in the cytoplasm more often than expected. Specific decavanadate interactions have been clearly demonstrated for myosin, calcium pump and actin, which are major proteins in muscle contraction and its regulation. Of particular interest are the V_{10} myosin back-door inhibition and the reduction of decavanadate by actin, although both processes still require to be clarified completely. Moreover, decavanadate inhibits strongly mitochondria, and therefore, cellular bioenergetics. In rat adipocytes, decavanadate can be a more potent insulin mimetic agent than BMOV, but preliminary results have shown a lack of effect in human adipocytes. It is proposed that the biological effects of vanadium may be explained, at least in part, by the capacity of decavanadate to induce many biological effects, some with medical applications.

In the present decade, we expect that important questions will be answered. (1) Will we be able to characterize the first X-ray structures of decavanadate-actin and decavanadate-myosin complexes? (2) Will we understand the role of decavanadate in the several steps of the process of actin polymerization/depolymerization? (3) Will we be able to understand the contribution of decavanadate as an insulin mimetic and anticancer agent? and (4) Will we be able to observe decavanadate formation in differ-

ent subcellular domains? These and others questions will require continuous development of new techniques and approaches to explore the vanadium effects in biology and their medical applications.

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