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Ascorbic acid in vanadium biochemistry, pharmacology and detoxification

Enrique J. Baran †

The most important aspects of the chemistry and structure of L-ascorbic acid (vitamin C) will be presented. Mechanistic and kinetic studies of the reduction of vanadium(V) by the acid are also discussed. Various oxidovanadium(IV)/ascorbato complexes could be prepared by different chemical reactions, the particular stability of the complex with 2,3-diketogulonic acid (one of the oxidation products of the acid) is emphasized. The participation of L-ascorbic acid in the treatment of diabetes or cancer and other diseases is also briefly discussed. The role of L-ascorbic acid in vanadium metabolism is of fundamental importance, as in natural systems it is one of the most important reducing agents of vanadium(V) species to oxidovanadium(IV) and can also stabilize the reduced species. It plays also an essential role in plant metabolism. Regarding vanadium detoxification, L-ascorbic acid appears to be the most effective detoxification agent for human use. It is probably the least toxic of all examined drugs and can be administered orally in relatively large doses. Brief commentaries are also made on other vanadium detoxification agents (for example, some well-known chelating agents and specially *meso*-2,3-dimercapto succinic acid and 2,3-dimercapto-1-propanedisufonate).

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Introduction

L-Ascorbic acid, often called vitamin C in a nutritional context, is naturally found in a wide variety of plants and animals. It is essential to man but is not produced in the human body and the only source is from diet. The structure of the acid has been determined by single-crystal X-ray crystallography.[1] It belongs to the monoclinic space group $P2_1$ with four formula molecules in the unit cell. The structure was later refined by neutron diffraction studies of single crystals and partially deuterated samples of the compound.[2] A most notable feature of this structure is the ene-diol arrangement. This structure produces acid–base behaviour such that the 3-hydroxyl is ionized first.

The acid acts as a good reductor for a wide range of metals and metal complexes but this redox behavior is complicated by the intervention of simultaneous proton transfer reactions,[3,4] as shown in Fig. 1.

Dehydroascorbic acid (Fig. 1, **2**) generated as the primary oxidation product of L-ascorbic acid 1, (**1**), is very unstable and undergoes a rapid series of transformations, as shown schematically in Fig. 1. It is degraded first to 2,3-diketogulonic acid (**3**) which can further be degraded to a mixture of oxalic acid (**4**) and L-threonic acid (**5**). At higher pH-values the latter acid is oxidized to tartaric acid (**6**).

L-Ascorbic acid also plays an essential role in the metabolism and other aspects of the chemistry and biochemistry of vanadium,[5] the most important ones shall be discussed in this article. In Table 1 the major events relevant to vanadium chemistry and biochemistry and L-ascorbic acid are chronologically summarized.

Kinetic and mechanistic studies of the reduction of vanadium(V) by L-ascorbic acid

L-Ascorbic acid can easily reduce vanadium(V) under different experimental conditions. The properties of the acid are strongly pH-dependent in aqueous solutions and as pH increases its reducing power increases. The ascorbate dianion is more readily oxidized than the monoanion, which in turn is more reactive than L-ascorbic acid.[6,7]

Various authors have investigated the most important and characteristic aspects of this process.

In 1973 Kustin and Toppen[8] determined experimentally that the stoichiometry of the reduction of vanadate(V) by L-ascorbic acid (H_2A) is: $H_2A + 2V(V) \rightarrow 2V(IV) + 2H^+$. The kinetics of the reaction was studied by stopped-flow procedures at 25 °C in acidic media (0.2–1.0 M $HClO_4$). The observed first-order rate constant is typical for the rapid formation of a complex followed by its rate-limited decomposition. On the basis of the obtained results it is

Centro de Química Inorgánica (CEQUINOR, UNLP, CONICET, CICPBA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 1900-La Plata, Argentina. E-mail: info_cequinor@quimica.unlp.edu.ar

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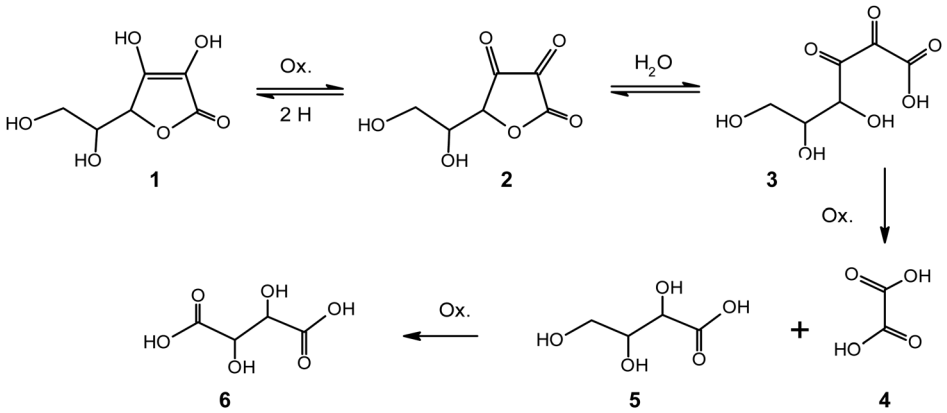

Fig. 1 Schematic representation of the stepwise oxidation of L-ascorbic acid.

Table 1 Chronology of milestones in the chemistry and biochemistry of vanadium and of L-ascorbic acid

1801	A. M. del Rio: discovery of vanadium
1830	N. G. Sefström: rediscovery of vanadium
1911	M. Henze: discovery of high levels of vanadium in the blood cells of the ascidia *Phallusia mamillata*
1927	A. Szent-Gyorgyi: discovery of L-ascorbic acid
1933	W. A. Harworth: structure determination of L-ascorbic acid
1936	H. Bortels: discovery of vanadium in nitrogen fixation
1972	E. Bayer: isolation of *amavadin* from the fly agaric (*Amanita muscaria*)
1983	H. Vilter: isolation of the first vanadium enzyme, vanadate dependent enzyme bromoperoxidase, in the marine alga *Ascophyllum nodosum*
ca. 1980	Development of vanadium compounds for the treatment of diabetes melitus
1986	Susex nitrogen fixation group: isolation of a vanadium nitrogenase from *Azotobacter*
ca. 2020	Development of vanadium compounds for cancer treatment

Is not clear at which year detoxification studies of vanadium were initiated.

plausible that the most probable mechanism is an inner-sphere, one-electron reduction of vanadium(v) by L-ascorbic acid.

Latter, the interaction was investigated over a wider pH-range (between 0.4 and 7.0) using a stopped flow system and the data analysis was complemented with the measurement of NMR and ESR spectra. Three different coordination complexes were detected. Both inner- and outer-sphere electron-transfers were proposed to form oxidovanadium(IV) species with L-ascorbate or dehydroascorbate respectively. Effects of the pH on the coordination of L-ascorbic acid to the vanadium center were observed and are surely related to the speciation of the vanadium(v) ion.

Three vanadium(IV) complexes were observed, analyzing the ESR data. Two of these complexes are proposed to be oxidovanadium(IV)/L-ascorbato species, and the other one is consistent with an oxidovanadium(IV)/dehydroascorbic acid complex. In the case of the ascorbato complexes a 1 : 2 species is formed at high ligand-to-metal ratios, whereas a 1 : 1 complex forms in solutions containing equimolecular ligand and metal ions. Finally, some mechanistic and stoichiometric aspects of all these reactions were also discussed.[9] This study gave also a good insight into the speciation of the vanadate/ascorbate system.

Other speciation studies were also performed. Ding et al. investigated the one-electron reduction of vanadate(v) by ascorbate, at physiological pH values by ESR and ESR spin trapping. The vanadium(IV) yield increases with increasing ascorbate concentration, reaching a maximum at a vanadium(v) : ascorbate ratio of 2 : 1. The vanadium(IV) generated by ascorbate reduction of vanadium(v) in the presence of phosphate was also capable of generating lipid hydroperoxide derivatives. Because of the ubiquitous presence of ascorbate in cellular systems at relatively high concentrations, reduction of vanadium(v) by ascorbate together with phosphate may represent an important vanadium(v) reduction pathway *in vivo*.[10]

Besides, Horton et al. investigated the reductive action of ascorbate on a dioxovanadate dipicolinate complex. Also, in this case the formation vanadium(IV) is observed. But, interestingly, in acid solutions also the generation of V(III) species were found, a result which may be relevant for vanadium in biological systems.[11]

Some other, more qualitative studies, of the system V(v)/L-ascorbic acid were also performed. They also gave other insights into the chemical behavior of this system. It has been tried to obtain solid samples of VO^{2+}/ascorbate complexes for a better characterization of these complexes. Following a general procedure developed by Jabs and Gaube[12] for the synthesis of transition metal ascorbate complexes it was possible to prepare two oxidovanadium(IV) complexes at different pH values. The obtained complexes respond to the formulas $[VO(HAsc)(OH)(H_2O)_2]\cdot H_2O$ (obtained at pH = 3.0) and $Na_2[VO(HAsc)_2(OH)_2]$ (obtained at pH = 7.0). In these complexes the acid acts as a monodentate ligand, generating species of very low stability, in agreement with absence of chelate binding. They were characterized by electronic, IR spectroscopy and thermogravimetric (TG/DTA measurements under a O_2 flow) analyses.[13]

It was also attempted to isolate oxidovanadium(IV) complexes of dehydroascorbic acid. Dimeric dehydroascorbic acid was prepared

by oxidizing ascorbic acid with *p*-benzoquinone in *N*,*N*-dimethylacetamide solution in the presence of an excess of concentrated formic acid and phthalic anhydride.[14] The interaction of dehydroascorbic acid with VO^{2+} was investigated at different pH-values. In all cases it was found that not stable dehydroascorbic complexes are generated, probably due to the instability of this species which oxidizes rapidly. Finally the formation of a complex with the enolized form of diketogulonic acid could be established.[13]

In another series of experiments sodium metavanadate were directly reacted with ascorbic acid.[15] It is usually admitted that in acidic media, metavanadate transformed easily to decavanadate (*cf.* for example[16,17]). In our experiments with ascorbic acid we have never observed this transformation. It was found that the nature of the generated complexes not only depends on the initial ratio L-ascorbic acid/metavanadate ratio but also on the used alkali hydroxide to adjust the final pH value. The pH of a mixture of the acid (40 mL of a 1 M solution) and metavanadate (40 mL of a 0.1 M solution) in aqueous solution was adjusted to neutrality with a 0.1 M KOH solution. By slow addition of acetone to this solution a green powder could be precipitated, which composition was determined as $K_{1.5}Na_{05}[VO(HAsc)(OH)_3]$. This complex is relatively unstable and the acid is bounded in a monodentated way.[15]

By mixing equimolecular 0.05 M solutions of both reagents and proceeding in the same way as above, a complex of composition $K[VO(diketo)(OH)]\cdot H_2O$ is obtained. Proceeding in a similar way as in the first case, with the only difference that the final pH neutralization was accomplished by dropwise addition of a 0.1 M NaOH solution, a complex of composition $Na_3[VO(diketo)_2(OH)]$ is obtained.[15]

The three complexes were characterized by magnetic susceptibility measurements and UV/visible and electronic diffuse reflectance spectra. On the other hand, the IR spectra clearly support the structural characteristics of the complexes. In $K_{1.5}Na_{05}[VO(HAsc)(OH)_3]$ one ascorbate moiety is bonded to the cation by its deprotonated 3-OH group and the coordination sphere is completed, generating a complex with a square-pyramidal geometry, with the cationic oxo-group on the apex of the pyramid. In $K[VO(diketo)(OH)]\cdot H_2O$ only one diketo group is present and the equatorial plane of the pyramid is completed by one OH^- group and the water molecule. In the third complex, the additional OH^- moiety is in *trans* position to the oxo group.[15] A schematical representation of this complex is shown in Fig. 2.

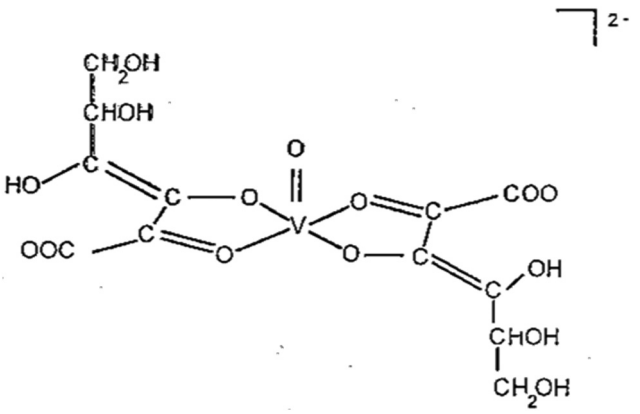

Fig. 2 Proposed structural model for the complex anion generated by interaction of VO^{2+} with 2,3-diketogulonic acid.

Additionally, TG and DTA measurements (working under an O_2 flow and at a heating rate of 10 °C min^{-1}) were also performed, not only to investigate the thermal stability of the three complexes but also to give an additional support to the proposed stoichiometries. The obtained results are clearly coherent with these proposals.[13]

The structure of the $Na_3[VO(diketo)_2(OH)]$ complex, without the presence of the OH^- group is shown in Fig. 2.

Vanadium/L-ascorbic acid systems with pharmacological properties

Only a very limited number of studies concerning pharmacological activity of the vanadium/ascorbic acid system have so far been reported. Notwithstanding, there are a number, generally recent, studies covering this aspect.

Proximal spinal muscular atrophy (SMA), a neurological disorder that causes infant mortality, has no effective treatment. It was recently shown that sodium vanadate has a good potential for the treatment of SMA. However, vanadate-induced toxicity *in vivo* remains an obstacle for its clinical application. The effects of the combination of sodium vanadate with the vanadium detoxification agent L-ascorbic acid, was investigated in an adult SMA mouse model. It was found that vanadium accumulation in kidneys and livers were largely reduced, and those organs retained normal function during development and adulthood. This work demonstrates that early treatment with vanadate combined with L-ascorbic acid has considerable potential for treating patients with SMA.[18]

A number of recent clinical studies with diabetic adult patients have demonstrated that the supplementation of ascorbic acid, alone or combined with conventional diabetic medication, can reduce blood glucose, increase insulin synthesis and secretion, improve insulin resistance, and reduce the development of complications of type 2 diabetes mellitus in these patients.[19]

It is known that exogenous insulin does not prevent cardiac failure in patients with type 1 diabetic mellitus and a cardioprotective insulin mimic is greatly needed. In a recent study the complex *cis*-bis(ascorbic acid)dioxomolybdenum(IV) was assayed for the treatment of diabetic mellitus type 1 and to overcome cardiac problems. Experiments were performed with streptozocin-treated rats. Once diabetes was confirmed, rats were treated during six weeks with the molybdenum complex or with sodium ascorbate. Following the treatment rats were euthanized with an injection of sodium pentobarbital. Blood samples were also obtained at this moment for analysis. Hearts were mounted in a hearth working perfusion apparatus to study its activity and general behavior. This study demonstrates a series of benefits of the administration of the molybdenum/ascorbic acid complex. It appears capable of combatting some of the symptoms of hyperglycaemia, strengthening cardiac diabetic function. It also suggests that the complex could be used in conjunction with regular antidiabetic treatments to

alleviate some of the cardiovascular problems often associated with diabetes.[20]

Sakurai and coworkers have found that oxidovanadium(IV)-*meso*-tetrakis(1-methyl pyridinium-4-yl) porphyrin (VOTMpyP) have a potent insulin mimetic activity on the basis of *in vivo* and *in vitro* experiments. It was found that it significantly lowers the blood glucose levels in STZ-rates. It was also found that when the complex is given simultaneously with sodium ascorbate, the high glucose levels of diabetic STZ-rats are lowered by synergistic effect. This was the first finding on not only the insulino mimetic activity of VOTMpyP but also the occurrence of a synergistic effect of sodium ascorbate to lower the high blood glucose levels in diabetic animals. This constitutes, evidently, a different pharmacological mechanism of ascorbic acid or its salts.[21]

Another interesting synergistic effect was recently observed, studying the binding characteristics of the transport protein bovine serum albumin (BSA) with levonorgestrel (LVG), an emergency contraceptive pill. It was found that ascorbic acid, and also salicylic acid, have an important effect on the binding behaviour of this system. Multi-spectroscopic techniques and molecular docking showed a high stability of the BSA–LVG complexes. LVG-induced changes in the BSA structure. The presence of ascorbic and salicylic acids seems to influence BSA conformation, reducing the stability of the BS–LVG complexes.

Serum proteins generally help to transport and distribute drug molecules within the body. In this study, the binding characteristics of bovine serum albumin (BSA) with levonorgestrel (LVG), an emergency contraceptive pill, and the influences of ascorbic acid (ASC) and salicylic acid (SAL) on the binding behaviour and protein structure were elucidated using multi-spectroscopic techniques and molecular docking. The results showed that levonorgestrel decreased BSA intrinsic fluorescence *via* static quenching mechanism. Binding constant (K_a) values for BSA–LVG complexes were 10^3 to 10^4 M^{-1}, indicating their high stabilities. Site probing/docking analysis indicated LVG bound between BSA subdomains IIA and IIIA. UV-visible absorption, Fourier transform-infrared and 3D fluorescence spectroscopies affirmed LVG-induced changes in BSA structure, especially in α-helix and β-sheet contents. ASC and SAL influenced BSA conformation for LVG binding and reduced the K_a values by 3.37 and 5.43-folds, respectively. LVG altered the microenvironments of tyrosine residues, and interacted with Arg-217, Lys-221, Val-292, Glu-443, *etc.* within the binding domains. The findings of the study offered details of the binding interaction between BSA and LVG, and also indicated that prior intake of ASC or SAL could suppress the binding affinity of BSA for levonorgestrel.[22]

At present there is not clear evidence that ascorbic acid alone can cure cancer, but in recent years numerous authors have explored the possible benefits of the acid in cancer treatments or to reduce side effects in chemotherapy and radiation therapy. Notwithstanding, high-dose ascorbic acid has been investigated as a treatment for cancer patients since more than forty years ago. Ascorbate acts as a pro-drug for H_2O_2 formation, and, through this mechanism, kills cancer cells. To achieve high *in vivo* doses, it must be injected by i.v. route. Recent clinical studies have shown significant anti-tumor activity against different cancer types. For example, it was found that ascorbate treatment of pancreatic cancer cells acted as a radiosensitizer of these tumoral cells to the DNA oxidative-damage induced by ionizing radiation. Some studies have also been carried out in ovarian cancer. It was shown that a high dose of ascorbic acid causes cancer cell death. The combination of parenteral ascorbic acid with conventional chemotherapeutic agents, for example carboplatin or paclitaxel synergistically inhibited ovarian cancer in mouse xenograft models and reduced-chemotherapy-associated toxicity in patients with ovarian cancer. Also some type of colorectal cancer cells were sensitive to the cytotoxic effects induced by high-dose ascorbic acid. The sensitivity of leukemic cells to the effects of the acid has also been explored. *In vitro* studies have shown that leukemic cell lines, particularly myeloid cell lines, are highly sensitive to the cytotoxic effects induced by pharmacological doses of ascorbic acid (*i.e.*, in the mM range). Ascorbic acid also has several biological properties allowing restoring the deregulated epigenetic response observed in many tumors.[23] Some of the commented effects were also supported by different studies of other authors.[24–28]

It is very important to remark that also decavanadate show an interesting antidiabetic potential probably related to the glucose uptake in adipocites.[29] Nutritional studies suggested that both vitamin C and vitamin B supplementation may be useful for cancer prevention and treatment, as various epidemiological studies indicate that deficiency of these micronutrients is associated with an increased risk and poor prognosis of several types of cancer.[30]

A specially interesting study was that of the osteoblastic activity of the complex of oxidovanadium(IV) with ascorbic acid (prepared according to ref. 15). At doses between 2.5 and 25 μM the complex significantly stimulates osteoblastic proliferation in UMR106 cells (rat osteosarcoma cells) but not in the MC3T3E1 cells (mouse-calvaria-derived cells). At doses between 5 and 100 μM the complex stimulates type-I-collagen production in osteoblasts. Because vanadate and other vanadium compounds are potent inhibitors of phosphatases, the possible effect of the complex on alkaline and neutral phosphatases were also investigated. It was found that it has a significant inhibitory effect with a dose dependent pattern. Maximal inhibition occurred at 100 μM concentration. All performed *in vitro* studies suggest that this oxidovanadium(IV) complex should be a useful pharmacological tool for bone tissue regeneration.[31] There are additional vanadium compounds made with molecules of therapeutic properties, although some of them are not approved by regulatory authorities. They include oxidovanadium(IV) flavonoids, which are active on different types of cancer[32] as well as numerous other vanadium complexes.[33]

L-Ascorbic acid in vanadium metabolism and detoxification

In living organisms, usually both vanadium(V) and vanadium(IV) species are present in serum.[34,35] In natural

systems, L-ascorbic acid is one of the most important reducing agents of vanadium(V) species to oxidovanadium(IV) and can also stabilize the reduced species. The other very important reducing agent that participates in vanadium metabolism is reduced glutathione (GSH), the tripeptide γ-L-glutamyl-L-cysteinyl glycine. Also this ligand can stabilize the generated reduced species. The oxidation product of glutathione (GSSG) can also interact with the VO^{2+} cation, and model speciation calculations reveal that in the pH-range 6.0–7.0 GSSG is a more effective oxidovanadium(IV) binder than GSH.[5]

Besides, the sulfur containing amino acid L-cysteine is another potential reduction agent for vanadates in biological systems. A study of the system VO_3^-/L-cysteine shows that vanadate is rapidly reduced, irrespective of the pH of the solution, followed by the formation of a purple complex. In this complex the VO^{2+} cation apparently interacts with the amino N-atom and the deprotonated –SH group of two amino acid molecules, generating a 2 : 1 (ligand to metal) species.[36]

Final excretion of the small fraction of absorbed and not retained vanadium mainly occurs through urine, as low molecular VO^{2+} complexes.[28] It was initially speculated that vanadium diascorbate is one of these species,[37] but on the basis of all the so far accumulated knowledge we think that probably the VO^{2+} complex of 2,3-diketogulonic acid (*cf.* Fig. 2) is the predominant species.

Besides, it is worth mentioning that L-ascorbic acid also plays an essential role in plant metabolism as oxalic acid (Fig. 1), one of its oxidation products, generates some of the most important and characteristic plant biominerals.[38–41] L-Ascorbic acid has the potential to be a good substrate for oxalic acid formation because it is present at relatively high levels in all higher plant species. Its synthesis occurs through the so-called Wheeler–Smirnoff mechanism.[42] Notwithstanding, it is not totally clear if the oxidation sequence presented in Fig. 1 is also involved in this way in the cleavage of ascorbic acid in plants, as also L-galactose is just as effective as ascorbic acid in oxalic acid biosynthesis.[42]

L-Ascorbic acid in vanadium detoxification

Environmental contamination by vanadium has dramatically increased during the last decades, especially in the most developed countries, due to the widespread use of fossil fuels, many of which liberate finely particulate V_2O_5 to the atmosphere during combustion.[43,44] Therefore, and also owing to the increasing interest in the pharmacological effects of some of its compounds, the toxicology and detoxification of vanadium constitute areas of increasing interest. In previous papers we have analyzed the most relevant aspects of vanadium toxicology and detoxification.[34,45]

The degree of vanadium toxicity depends on the route of incorporation, valence and chemical form of the element, and is also, to some extent, species dependent. In general, it increases as valence increases, pentavalent vanadium being the most toxic. Although under normal natural conditions, toxic effects do not occur frequently, at high doses or as a consequence of chronic exposure, it is a toxic element for humans.[34,46,47] The upper respiratory tract is the main target in occupational exposure. Vanadium compounds, especially V_2O_5, are strong irritants of the airways and eyes. Acute and chronic exposure gives rise to conjunctivitis, rhinitis, and to bronchitis, bronchospasms and asthma-like diseases in more severe cases. It can also produce fatigue, cardiac palpitation, gastrointestinal distress, kidney damage and even neurological disorders. In humans acute toxicity has been observed in vanadium miners and industrial workers exposed to high doses of vanadium. The classic symptoms of this malady, referred to as "green tongue" syndrome are a green coloration of the tongue, accompanied by some of the above mentioned-disorders.[34,46]

It is very important to remember that vanadium toxicity is not only related to the presence of vanadate(V) species, but also to that of minimum quantities of $V_{10}O_{28}^{6-}$ generated upon V_2O_5 solubilization in biological media (*cf.* for example ref. 48 and 49).

Regarding vanadium detoxification it can occur through biological mechanisms or by the use of adequate chemical agents. It is well known that all living organisms have developed defense mechanisms to deal with the reactive and potentially harmful by-products generated by cellular metabolism and to control the effects of exogenous substances that eventually invade the organism.[50] This is called biological detoxification. On the other hand, a series of drugs (usually chelating agents) that are capable of chelating metal ions *in vivo* have been developed not only to eliminate excess of essential metals but also to prevent possible damage caused by nonessential, toxic elements. This is the basis of the so called chelation therapies and constitutes the chemical detoxification ways.[51,52]

Biological detoxification of vanadium surely occurs through some of the mechanisms mentioned above, in relation with its metabolism, *i.e.*, redox processes involving glutathione or ascorbic acid and, eventually sugars or polysaccharides which interact with the metal acting as reductants and/or chelators.[34,45] The accumulation of vanadium in bone must also be considered in this context, as bone seems to be the most active vanadium accumulator. This high skeletal retention of vanadate is surely related to its rapid exchange with bone phosphate, which is favored by the strong similarities between VO_4^{3-} and PO_4^{3-}.[34,53,54]

Chelation therapy occupies a central place in modern medicine and pharmacology as extensive clinical experiences and studies with laboratory animals demonstrate that acute or chronic human intoxication with a variety of metals can be considerably improved by administration of suitable chelating agents. In the case of vanadium, most of the so far known and well-established chelating agents employed in the clinical praxis have been tested, with varying success, for vanadium detoxification, generally with laboratory animal experiments.[34,45] Well-known chelating agents such as ethylenediaminetetraacetic acid (EDTA), and related polyaminopolycarboxylic acids, have been profusely investigated. EDTA, in particular, shows a good chelating behavior for both vanadium(V) and vanadium(IV) species. Also for D-penicillamine a good activity against both vanadium species has been reported.[38] As demonstrated by animal studies, also desferrioxamine B, one of the best known siderophores and

a widely used chelating agent for the treatment of iron overload conditions,[52] has been shown to be a very effective antidote for vanadium poisoning. It raises urinary and fecal vanadium excretion and is effective in the removal of both vanadate and oxidovanadium(IV) species.[45]

From all the vanadium detoxification agents investigated so far, L-ascorbic acid appears to be the most effective for human use, as shown by an important number of studies.[34,55–58] It is probably the least toxic of all examined drugs and can be administered orally in relatively large doses.[34] Its strong detoxification activity can surely be related to the facility with which it reduces vanadium(V) to VO^{2+}. As this oxocation does not form stable complexes with the acid and, as discussed above, the elimination route surely involves one of the oxidation products of the acid as ligand, most probably 2–3, diketogulonic acid.

During the last decades two new interesting chelating agents entered in the medical practice. They are *meso*-2,3-dimercapto succinic acid (DMSA, Fig. 3A) and 2,3-dimercapto-1-propanesulfonate (DMPS, Fig. 3B).

The two diol groups present in both drugs generate an important reducing power, which is stronger in the case of DMPS. Besides, both drugs are less toxic than other usual chelating agents and can be administered orally or parenterally. DMSA is usually employed directly in the form of the free acid whereas DMPS is commercialized in the form of its monohydrated sodium salt.[52] The action of these two new chelating agents on VO^{2+} and other vanadium species was investigated.

The interaction of the VO^{2+} cation with meso-2,3-dimercaptosuccinic acid (DMSA) was investigated by electron absorption spectroscopy in aqueous solutions at different pH values. The spectral behaviour, complemented with a spectrophotometric titration, shows the generation of a $[VO(DMSA)_2]^{2-}$ complex in which the oxocation interacts with two pairs of deprotonated –SH groups of the acid. To verify the reducing action of DMSA over vanadium(V) species, its interaction with $NaVO_3$ and V_2O_5 was also investigated. In the experiments with vanadate a very rapid reduction of vanadate(V) to oxidovanadium(IV) was observed. In the case of the pentoxide the reaction is much slower, but gradually it transforms also to the $[VO(DMSA)_2]^{2-}$ complex.[59] A similar study with 2,3-dimercapto-1-propanesulfonate (DMPS) gave identical results.

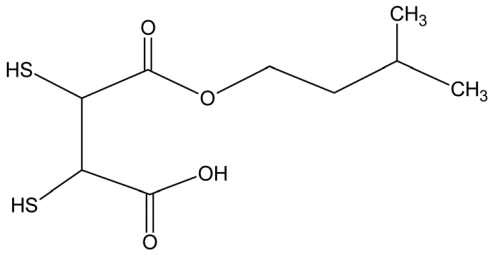

Fig. 4 Schematic structure of the monoisoamyl ester of DMSA (MiADMSA).

Interaction of VO^{2+} with DMPS, in the pH range between 4 and 12, generates the $[VO(DMPS)_2]^{4-}$ complex in which the interaction of the oxocation occurs again through two pairs of deprotonated –SH groups of the ligand. As in the previous case, it was found that DMPS rapidly reduced vanadates(V) to VO^{2+} which may be chelated by an excess of the acid. Besides, suspensions of V_2O_5 are also reduced very slowly by DMPS.[60]

Although DMSA is a very effective and potent chelating agent, because of its hydrophilic and lipophilic properties it is unable to pass through cell membranes, and as a consequence, it cannot capture intracellularly deposited toxic species. Thus, a large number of esters of DMSA have been synthesized to overcome this problem and to enhance tissue uptake. In order to make the compounds more lipophilic, the length of the carbon chain of the parent DMSA was increased by controlled esterification with different alcohols (methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl and hexyl). Most of these esters have been assayed for the treatment of metal poisoning, and it has been reported that they have better potential than DMSA for the mobilization of toxic metals (*cf.* for example ref. 61). Among these new chelators, the monoisoamyl ester of DMSA (MiADMSA, Fig. 4) has been found as particularly effective.[52]

MiADMSA was synthesized and its interaction with VO^{2+} investigated by electron absorption spectroscopy in the pH range between 6.3 and 11. The formation of a complex species of stoichiometry $[VO(MiADMSA)_2]^{4-}$ in which the oxocation intracts with two pairs of deprotonated –SH groups of the ester, was demonstrated. Besides, MiADMSA rapidly reduced both VO_3^- and V_2O_5 to oxidovanadium(IV).[62]

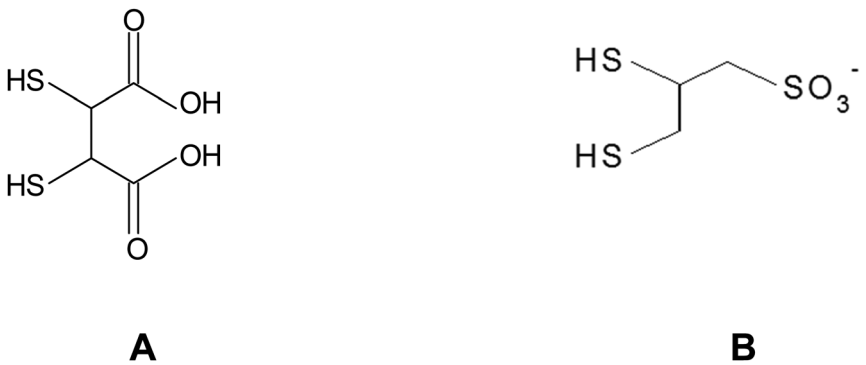

Fig. 3 Schematic structures of *meso*-2,3-dimercapto succinic acid (DMSA, (A)), and 2,3-dimercapto-1-propanesulfonate (DMPS, (B)).

The results of these studies clearly show that DMSA, DMPS and MiADMSA are very promising and interesting detoxification agents for biologically relevant vanadium species (Table 2).

Table 2 Major issues highlighted in this Review

Structure of L-ascorbic acid
Redox behaviour of L-ascorbic acid
Kinetic and mechanistic studies of the reduction of vanadium(V) by L-ascorbic acid
Speciation in the vanadate/ascorbate system
Synthesis of VO(IV) complexes of L-ascorbic acid and its oxidation products
Pharmacological properties of L-ascorbic acid
Antidiabetic and antitumoral action
Synergistic effects of L-ascorbic acid
Osteoblastic activity of the VO(IV)/ascorbate complex
Reducing agents of vanadium(V) in biological systems
L-Ascorbic acid in plant metabolism
L-Ascorbic acid in vanadium detoxification
Chelation therapies. New chelating agents used in vanadium detoxification

Conclusions

As shown in this study, L-ascorbic acid plays a very important role in natural vanadium metabolism and detoxification. Its participation in some antidiabetic and antitumoral systems opens new interesting ways in pharmacology. It is also the most important vanadium detoxification agent for humans, as it is not toxic and can administered orally in relatively large doses. It is evidently, that it is important to extend and deepen our knowledge on the ascorbic acid/vanadate interactions as well as on the joint role of those actors in biological systems.

Conflicts of interest

There are no conflicts to declare.

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