Vanadium uptake by higher plants: some recent developments

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ABSTRACT

The occurrence of vanadium in the biosphere, and the possible roles this element may play in the metabolism of living organisms, especially higher plants, are discussed. The aqueous chemistry of the element is reviewed, and the chemical properties of the element are related to those of soils and plants. Evidence is present for a biotransformation of vanadium from vanadate (VO_3^-) to vanadyl (VO^{2+}) during uptake by plants, based on tissue analysis and ESR spectra. The significance of this process on the potential impact of vanadium in the biosphere is discussed.

VANADIUM IN THE BIOSPHERE

Vanadium is a ubiquitous element in the biosphere, and trace levels are detectable in most living organisms. However, the role it plays in the metabolism of these organisms is currently a matter for debate. Vanadium has been recognised as an essential element for certain species of algae (Arnon and Wessel, 1953), but there is little evidence to support claims for its essentiality for higher plants. Welch and Huffman (1973) demonstrated that both lettuce and tomato could be grown to full maturity in media containing less than $4 \mu g.L^{-1}$ of vanadium. Hewitt (1966) grew sugar beet, tomato, lettuce, alfalfa and clover to full maturity in media containing less than $2-3 \mu g.L^{-1}$ vanadium. Any requirement for vanadium in the metabolism of higher plants must be of a minute order of magnitude.

Circumstantial evidence has been offered to support the concept of the essentiality of vanadium in animals (Hopkins, 1974); some authors have even suggested it may be essential for man (Nielsen and Sandstead, 1974). At present, there is no conclusive evidence to support either premise, although recent findings on the role of vanadium in the inhibition of Na-K ATPases may prove to be of significance in all aspects of active metabolism.

There is evidence for significant increases in the release of vanadium into the biosphere over the last few decades. Vanadium emissions, mainly due to the combustion of fossil fuels, are now equal, on a global scale, to natural emissions into the atmosphere from continental dust, marine aerosols and volcanic activity (Zoller *et al.*, 1973). It is estimated that the amount of vanadium released into the atmosphere from anthropogenic sources exceeds total world production of the metal (Vouk and Piver, 1983). There are reports of direct plant damage caused by contact with vanadium-rich ash (Vaccarino *et al.*, 1983), and there is every possibility of this being repeated in the future.

AQUEOUS CHEMISTRY OF VANADIUM

Vanadium possesses an extremely complex aqueous chemistry. It is a Group V transition metal which exhibits multiple oxidation states, the four most frequent being +2, +3, +4 and +5. The latter 3 are the only forms with any biological significance. The +3 oxidation state only occurs in one group of marine chordates; the +4 and +5 are the most significant oxidation states in living organisms.

In aqueous solution, V(5+) exists as a number of oxoions known as vanadates. The nature of vanadate species present in solution depends on both pH and concentration. Moreover, since equilibrium between the various species is slowly attained, the exact species present cannot be stated with certainty unless the solution is allowed to stand for several days. For a more detailed discussion of the problems see Chasteen (1983).

In aqueous solution, the chemistry of tetravalent vanadium is centred around the VO^{2+} ion. In the absence of strong complexing ligands, this undergoes a number of hydrolysis reactions with increasing solution pH. These can be described by the following equations:

$$VO^{2+} + H_2O \rightarrow VOOH^+ + H^+$$
$$2VO^{2+} + H_2O \rightarrow (VOOH^+)_2 + 2H^+$$
$$VO^{2+} + 2OH^- \rightarrow VO(OH)_2$$
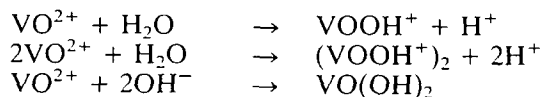
At pH < 5.0, VO^{2+} and $VOOH^+$ dominate, but at more alkaline pHs, a diversity of ionic forms is evident. It must also be noted that all these ionic forms are susceptible to air oxidation especially in an alkaline environment. This oxidation will however be retarded if a large percentage of hydrolysed species, or suitable chelating agents, are present.

Both oxidation states of vanadium have a marked tendency to form stable chelates with a wide variety of organic and inorganic ligands. The most stable chelates are formed with organic ligands such as ketones,

aldehydes, catechols, amino compounds and phenols. Often, the formation of a stable chelate alters the oxidation state of the vanadium, for example when V(5+) oxidises ascorbic acid or cysteine to produce V(4+).

GEOCHEMISTRY AND SOILS

Vanadium has a mean crustal abundance of 150 mg kg^{-1}. This places it fourteenth in the order of geochemical abundance, ranking alongside such essential elements as cobalt, copper, molybdenum and zinc (Hansen, 1983). Vanadium is more widely dispersed than these elements, therefore in principle it is more readily accessible by plants than those elements which tend to be concentrated in discrete bodies. Soil levels are dependent on the parent material, but generally range between 10–150 μg g^{-1} d.wt (Table 1). Extractable vanadium levels are related to the extractant used; these are generally low (<5 μg g^{-1}) when acetic acid or ammonium acetate are the extractants used but may be elevated if an organic extractant such as EDTA is used.

Whilst many workers have given consideration to soil vanadium levels, few have considered the form in which this element is mobilised. Vanadium in the earth's crust exists in a number of valence states in a variety of minerals. It is generally accepted that, during soil formation, reduced vanadium is fully oxidised to the pentavalent state. The oxyanions of this latter form are soluble over a wide pH range and are thought to represent the mobile form of vanadium in soil. The extent of any mobility is determined by edaphic factors, notably the presence of reducing agents. Several workers have demonstrated the ability of soil organic matter to reduce pentavalent vanadium to the tetravalent state (Szalay and Szilagy, 1967; Goodman and Cheshire, 1975), and it may be possible that vanadium is mobilised in soils as a cationic complex.

VANADIUM IN PLANTS

Several authors have presented compilations of plant tissue vanadium levels (Bengtsson and Tyler, 1976; Bertrand, 1950; Hopkins et al., 1977). A figure of 0.5–2.0 μg g^{-1} on a dry weight basis is generally agreed for plants from unpolluted sources. Elevated vanadium levels have been reported from plants growing in the highly mineralised soils of the Colorado plateau, U.S.A., with species of the genus Astragalus containing up to 144 μg g^{-1}. Even some non-accumulator plants were found to contain elevated V levels when cultivated in close proximity to highly mineralised soils (Table 2).

Early observations relating to vanadium uptake by higher plants arose from studies primarily concerned with other elements. Brenchley (1932), in experiments on phosphate fertilisation, found that fine grinding of the fertiliser tended to reduce yield. This was attributed to a toxic agent, subsequently identified as vanadium. Later work indicated that levels of 1–2 μg g^{-1} vanadium in the nutrient solution could produce reductions in shoot biomass. Experiments carried out by Warington (1951, 1954) demonstrated that vanadium could relieve symptoms of excess Mn in soyabean, but this treatment caused symptoms of vanadium toxicity (apical chlorosis and abnormal root growth). This toxic response could be counteracted by increasing the supply of Fe. Wallace et al. (1977) demonstrated a reduction in dry matter production in plants grown in the presence of 10^{-4} M vanadate. In these experiments, tissue V levels increased in relation to the rate of supply; the major part of this remained in root tissues. This was also noted by Hara et al. (1976), who found retention of vanadium in cabbage roots at levels up to 2500 μg g^{-1} dry wt. This represented 95–98% of the total V absorbed.

Recent experimental studies

Work in this laboratory has concentrated on the mechanisms of uptake of the different chemical forms of vanadium, in both whole plants and excised plant roots. In the experiments described below, vanadium was supplied as either vanadyl sulphate (VO^{2+}) or ammonium metavanadate (VO$_3^-$). Initial studies on whole plants examined the uptake of these two forms over a range of concentrations. Experiments with maize (Zea) showed an increased uptake of both oxidation states as the rate of their supply increased, with the pattern of retention by the roots, as observed by other workers, readily apparent. Relationships between root and shoot vanadium levels following its supply in either of the ionic forms could be described by a regression line. This relationship is depicted in Figure 1, and a comparison of the regression lines for both ionic forms reveals no significant differences (Table 3). Further experiments with broad beans (Vicia) revealed a very similar picture; the bulk of applied vanadium (97%) was retained by the roots, this pool increased in proportion to the rate of V supply, irrespective of chemical

Table 1 Vanadium levels in a variety of soil types.

Soil type	Concentration μg g^{-1} dry wt.)
Tundra soil	105
Podzol	66
Chernozem	107
Forest soil	73
Spruce mor, purely organic topsoil	4–11
Scottish peaty podzol	97
Surface soil polluted by fly ash	30–429

Table 2 Unusually high concentrations of vanadium in plants grown in the U.S.A. in soils containing high concentrations of vanadium (dry weight) from Hopkins et al. (1977).

Species	Plant part	V (μg g^{-1})	Area
Triticum aestivum	aerial	5	U.S.A.
Allium macropetalum	entire	133	Utah
Oenothera caespitosa	aerial	38	New Mexico
Astragalus confertiflorus	aerial	144	Utah
Astragalus preussi	aerial	67	Utah
Aster venustus	aerial	21	Utah
Castilleja angustiflora	aerial	37	Utah
Chrysothamnus viscidflorus	aerial	37	Utah
Eriogonum inflatum	aerial	15	Utah
Lepidium montanum	aerial	11	Utah
Mielichhoferia	entire	154	Alaska

form. The relationships between root, stem and leaf vanadium levels could be compared with each other by linear regressions (Figure 2). Once again, there was no significant difference between either of the ionic forms of vanadium (Table 4).

The similarity in both uptake and transport of these two different ionic forms leads to speculation that biotransformation of vanadium may be occurring during uptake by whole plants. Vanadium (+4) has an unpaired electron, making this ion paramagnetic and capable of producing an ESR (electron spin resonance) signal. Vanadium (+5) has all electrons paired and will not give an ESR signal. In view of this, experiments were conducted to investigate changes in ESR signals produced by the different forms of vanadium during uptake by whole plants. Barley seedlings were germinated, then grown in 0.5mM $CaCl_2$ solution containing

Figure 1 *Regression lines describing the relationship between root and shoot vanadium levels in* Zea *when supplied with either (a)* VO^{2+} *or (b)* VO_3^-.

Table 3 *Equations of the regression lines depicted in Figure 1, and a comparison of the regression coefficients for both lines.*

	(a) VO^{2+}	(b) VO_3^-
	$r^2 = 0.68$	$r^2 = 0.74$
	$Y = 0.019x - 0.022$	$Y = 0.015x - 1.58$

Comparison of regression coefficients		
n	41	41
sum $(x - x)^2$	4.60×10^7	3.81×10^7
variance	202.23	79.86
b	0.019	0.015
	$d = 1.154$ not significant $(P < 0.05)$	

(d ratio allows simple comparison of two regression coefficients, see Bailey (1974).)

Table 4 *Comparison of regression coefficients for relationships between various tissues of bean (from Figure 2).*

Root-leaf regressions:

	VO^{2+}	VO_3^-
Sum $(x - \bar{x})^2$	8.02×10^6	7.09×10^6
Variance	19.15	18.25
n	27	27
B	0.0062	0.0099
$F = 1.049$	NS Variances are equal.	
$d = 1.672$	NS No difference in B values.	

Root-stem regressions:

	VO^{2+}	VO_3^-
Sum $(x - \bar{x})^2$	8.021×10^6	7.092×10^6
Variance	2.65	4.51
n	27	27
B	0.0045	0.0049
$F = 1.700$	NS Variances are equal.	
$d = 0.453$	NS No difference in B values.	

Stem-leaf regressions:

	VO^{2+}	VO_3^-
Sum $(x - \bar{x})^2$	230.2×7	287.33
Variance	8.008	27.8
n	27	27
B	1.592	1.606

Variance comparison $F = 3.48$ variances significantly different.
As the regression lines have unequal variances, a modified number of df must be calculated, in this instance df = 41, d = 0.04 no difference in B values.

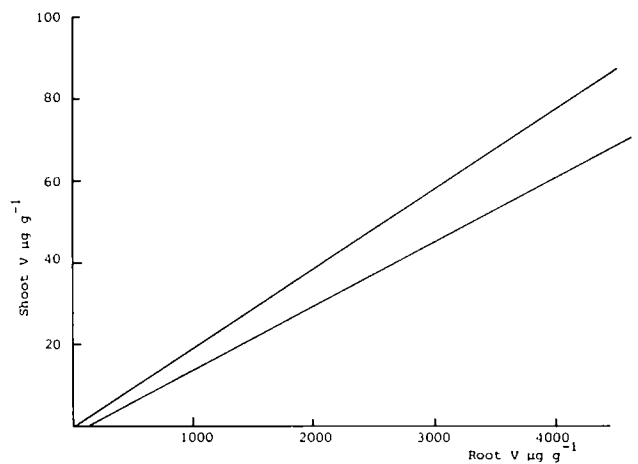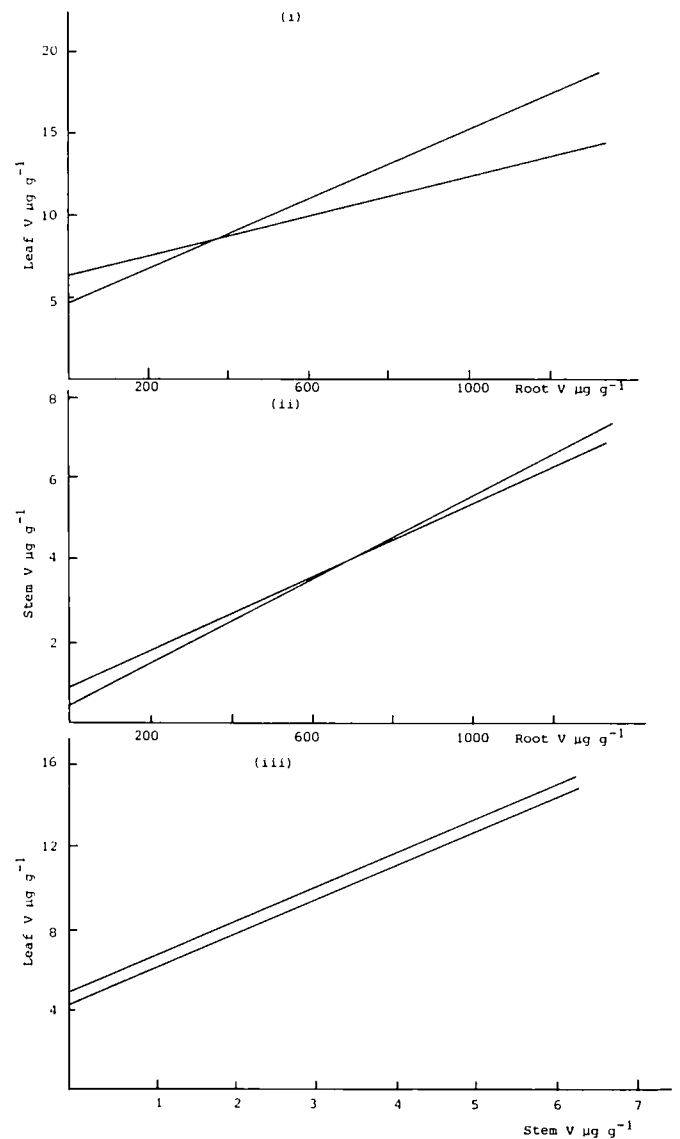**Figure 2** *Regression lines describing the relationships between the vanadium content of different tissues of* Vicia *plants treated with either (a)* VO^{2+} *or (b)* VO_3^-. *(i) Root vs leaf, (ii) Root vs stem, (iii) Stem vs leaf.*

either $VOSO_4$ or NH_4VO_3 supplied at 0.1mM. Roots grown in $CaCl_2$ alone produced no ESR signal. Roots grown in the presence of $VOSO_4$ produced a characteristic eight peak ESR spectrum (Figure 3(a)). Roots grown in the non-paramagnetic NH_4VO_3 also produced an eight peak spectrum characteristic of vanadium (+4) (Figure 3(b)). These results strongly support the hypothesis of biotransformation of vanadium during uptake by plant roots. This process would seem to involve the reduction of pentavalent vanadium

although the extent of reduction is unclear. A mechanism has been proposed whereby this process might occur via the oxidation of acid groups of cell wall polysaccharides (Deiana *et al.*, 1983).

$$\begin{array}{c} \text{H} \quad \text{H} \\ | \quad | \\ -\text{C}-\text{C}=\text{O} + 4\text{VO}_3^- + 12\text{H} \rightarrow -\text{C}=\text{O} + 4\text{VO}^{2+} \\ | \qquad\qquad\qquad\qquad\qquad + \text{HCOOH} + 6\text{H}_2\text{O} \\ \text{OH} \end{array}$$

$$\downarrow$$

$$\text{V(IV) COMPLEX}$$

Even at pH 7, vanadium (+5) will readily oxidise aldehydes, ketones, catechols, olefins and sulphydryls, all commonly found in cell walls. This would indicate the potential for the ready reduction of vanadium by root tissues. Such a mechanism would also help to explain the strong retention of vanadium by root tissues; V^{4+} complexes resulting from the above process would be extremely stable and would certainly render the bulk of any free space vanadium physiologically unavailable.

The results discussed above strongly support the hypothesis that vanadium in plant tissues exists, at least partly, in the reduced tetravalent form. The extent of reduction is unclear but present results in no way preclude the possibility that pentavalent vanadium (+5) is also present in those tissues. This reducton occurs during the process of uptake into the free space of the root cortex, although the exact timing and location of this process is at present uncertain. Reduction is thought to lead to immobilization of vanadium within the root tissues and is likely to be the dominant factor in determining the availability of this element to aerial plant tissues.

The importance of this reduction of vanadium is apparent when the effect of the vanadate ion on Na-K ATPases is considered. Vanadate (+5) is recognised to be a potent inhibitor of several of these enzymes, which are largely unaffected by the reduced form of vanadium (+4) (Macara, 1980). Given the ubiquitous occurrence of Na-K ATPases in membrane systems, the effects of vanadate on all living systems are potentially catastrophic. Reduction represents a highly effective means of limiting potential disruption in these systems.

CONCLUSIONS

Despite recent efforts, vanadium still remains an enigmatic element; it undoubtedly has the potential to disrupt severely the metabolic integrity of living matter, yet its chemical properties, especially in the case of its interactions with common organic constituents of living matter seem to render its disruptive potential innocuous. It is to be hoped that this fortuitous state of affairs continues and that conditions which allow free access of vanadate ions to living systems are not created in the biosphere. The likelihood of this seems to be increasing and already the impact of increased atmospheric loading has been recorded (Vaccarino *et al.*, 1983). Vanadium's position as a 'low profile element' may soon alter.

a

b

Figure 3 *ESR spectra of intact barley plants treated with vanadium as either (a) VO^{2+} or (b) VO_3^-.*

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