ELSEVIER

Coordination Chemistry Reviews
219–221 (2001) 1033–1053

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

Coordination chemistry of vanadium in metallopharmaceutical candidate compounds

Katherine H. Thompson, Chris Orvig *

Medicinal Inorganic Chemistry Group, Chemistry Department, University of British Columbia, 2036 Main Mall, Vancouver, BC, Canada V6T 1Z1

Received 26 January 2001; accepted 25 May 2001

Dedicated to Professor A.B.P. Lever, with great affection and admiration, on the occasion of his 65th birthday; long may he edit

Contents

Abstract 1034
1. Introduction 1034
2. Inorganic pharmaceutical agents 1035
   2.1 Desirable properties of metallopharmaceuticals 1035
   2.2 Unique features of vanadium 1035
   2.3 Vanadium complexes: toxicity, potency and relative efficacy 1036
3. Vanadium-containing insulin enhancing agents 1037
   3.1 V(IV) insulin enhancing agents 1037
      3.1.1 Pyronates and pyridinonates 1037
      3.1.2 Picolinates 1038
      3.1.3 Acetylacetonates 1039
      3.1.4 Dicarboxylate ester–oxovanadium(IV) complexes 1040
      3.1.5 Peptide bound complexes 1041
      3.1.6 V(IV) SALEN derivatives 1042
      3.1.7 Sulphur-containing ligands 1043
      3.1.8 Hypoglycemic agents as ligands 1044
   3.2 V(V) protein-tyrosine phosphatase inhibitors 1044
      3.2.1 Mono- and di-peroxovanadates 1044
      3.2.2 Peroxo-complexes with amino acids and analogues 1045
   3.3 V(III) complexes 1046
4. Other vanadium-based therapeutic agents 1047
   4.1 Anti-neoplastic agents and chemotherapeutic complexes 1047

* Corresponding author. Tel.: +1-604-822-4449; fax: +1-604-822-2847.
E-mail address: orvig@chem.ubc.ca (C. Orvig).

PII: S0010-8545(01)00395-2

4.2 Vanadium compounds as spermicides and anti-inflammatories 1048
5. Putative mechanism(s) of vanadium's effects 1048
5.1 Advantages and disadvantages of oxidative stress 1048
5.2 Other considerations of mechanism 1049
6. Summary and conclusions 1049
Acknowledgements 1050
References 1050

Abstract

The discovery of vanadium's insulin-like behaviour in vitro, and later of the orally available glucose- and lipid-lowering capability of these same compounds in vivo, has stimulated renewed interest in vanadium coordination chemistry. Besides the anti-diabetic effects for which it is now so well known, vanadium also exhibits a number of other therapeutic effects including anti-tumour and anti-inflammatory activities. In this review, emphasis will be on the most recent developments in the coordination chemistry of vanadium(III), (IV) and (V), as related to development of these compounds for pharmaceutical use. How best to measure bioactivity and the pharmaceutical relevance of accompanying increased oxidative stress will also be considered.

Keywords: Vanadium complexes; Diabetes mellitus; Cancer; Pharmaceutical agents; ESEEM; Structure–activity relationships; Bioactivity

1. Introduction

Vanadium, element number 23, atomic weight 51 Da, exists in a variety of oxidation states, from -3 to $+5$ [1]. In vivo, given the constraints of standard physiological conditions (pH 3–7, aerobic aqueous solution, ambient temperature) oxidation states $+4$ and $+5$ prevail, with thermodynamically plausible species including vanadate, a mixture of $[HV^{V}O_4]^{2-}$ and $[H_2V^{V}O_4]^{-}$, and vanadyl, $V^{IV}O^{2+}$ [2]. Coordination complexes of vanadium which may have pharmacological relevance, however, include not only vanadate $[V^{V}O_xL_y]$ and vanadyl $[V^{IV}OL_Z]$ complexes, but also the peroxovanadates $[V^{V}O(O_2)(H_2O)(L\text{–}L')]^{n-}$, $n = 0,1$; and $[V^{V}O(O_2)_2(L\text{–}L')]^{n-}$, $n = 1, 2, 3$ [3].

In this review, the emphasis will be on recent developments in the coordination chemistry of vanadium relevant to all its potential medicinal uses. This will, of necessity, exclude a great deal of current research on other aspects of vanadium coordination chemistry, for which the reader is referred to a number of recent comprehensive reviews [4–7]. In addition, the focus will be on the coordination chemistry of the latest compounds having pharmaceutical potential which have been completely characterized structurally; for more complete coverage of other aspects of vanadium and diabetes [8,9], vanadium essentiality [10], and toxicity of vanadium in mammals [11], readers are directed to the suggested reviews, and to several complete volumes on vanadium and its biological role(s) [12–15].

2. Inorganic pharmaceutical agents

2.1. Desirable properties of metallopharmaceuticals

Desirable qualities of compounds intended for use as pharmaceutical agents include neutral charge, low molecular weight, thermodynamic and hydrolytic stability, oral bioavailability, and, where possible, bi-functional capability [16]. A candidate therapeutic agent must be able to cross biological membranes, both for the initial absorption process, and for intracellular uptake. Very few metal ions have inherent active or facilitative transport mechanisms, exceptions being essential metal ions such as copper, zinc and iron [17]. Vanadium's essentiality is still debatable, and it has no known active transport mechanisms in mammalian cells [18]. Most other non-essential metal ions are assumed to cross cell membranes by passive diffusion, which requires that the metal complex have low molecular weight and no positive or negative charge, as well as a fair degree of resistance to hydrolysis [19]. A high synthetic yield and known non-toxic metabolic products are also advantageous. Because biological membranes contain a high proportion of lipids, lipophilic complexes can be expected to cross cell membranes more readily than hydrophilic ones [20].

Ligands can potentially be tailored to modulate systematically redox potential, electron-transfer rate, and magnetic moment of the bound metal ion [21]. One can also theoretically predict the steric strain within coordination metal complexes [22] and modulation of electronic characteristics of the metal ion by complexation [23]. Other properties, such as solvation and stability of binding with commonly encountered biomolecules, may be harder to anticipate, based on empirical considerations [24].

An ability to tailor the physical properties of the ligand with regard to hydrophilic/lipophilic balance is of particular importance with respect to transport of metal ions across biological membranes [25]. A stronger affinity between ligand and metal would be expected to alter the in vivo substitution chemistry with endogenous ligands of both low molecular mass (e.g. citrate, ascorbate and glutathione) and high molecular mass (e.g. transferrin and albumin) [26]. An available strategy for improving biological functionality may be to include sulphur coordination for potentially useful enzyme binding in vivo [27], or to introduce carbohydrate moieties for preferred membrane transport [17].

2.2. Unique features of vanadium

Vanadium is one of the most redox active elements, and forms both cationic and anionic complexes in the pH range likely to be encountered physiologically (pH 2–8) [28]. In vivo, the key redox interplay is between V(V) and V(IV), with the two oxidation states coexisting in equilibrium both intra- and extra-cellularly [29]. Vanadium's redox balance is mediated in vivo by oxygen tension, acidity and the presence of endogenous reducing agents such as ascorbate, glutathione and catecholamines [5,28]. Although vanadium has been proposed as an in vivo regulator of

cell metabolism [2] (particularly with regard to Na^+, K^+-ATPase inhibition), it has as yet no proven biochemical function in mammals [10]. In certain marine organisms, vanadium is a required cofactor for a number of haloperoxidases [30]. In rats, an extremely low vanadium diet, coupled with high dietary iodine, results in clear aberrations in thyroid hormone metabolism [18]. In vitro, vanadium's effects on various components of the intracellular signaling cascade are manifold [31]. At higher concentrations (1–5 mM), vanadium's insulin-like stimulatory or inhibitory effects on specific glucose- and lipid-related enzyme systems have also been amply demonstrated [32].

From a coordination chemistry point of view, vanadium is remarkably flexible. V(V) has particularly non-rigid stereochemical requirements and can form coordination complexes in geometries ranging from tetrahedral and octahedral to trigonal- and pentagonal-bipyramidal. V(IV) is much less flexible, with square pyramidal or, if a sixth position is occupied, distorted octahedral geometries [6,7]. Complexes may show widely varying thermodynamic stability and kinetic inertness [33].

2.3. Vanadium complexes: toxicity, potency and relative efficacy

In common with other minerals, essential and non-essential, vanadium has a range of usual dietary intakes; a (not yet established) least observed adverse effect level (LOAEL [34], a toxicological measure of exposure); and an (intermediate) window of potential therapeutic effect, with a concentration range (or ranges) that may be disease-specific [10,34]. Complexation with ligands may alter this window, due to competitive binding, additional redox stability, or cooperative effects [35]. Overcoming vanadium's inherently low gastrointestinal absorption (generally less than 1% of an oral dose) [10] is an important avenue for increasing pharmacological potency, a measure of drug response for a given dose [36]. Another mode of decreasing the likelihood of toxic side effects, by preserving the oxidation state, may improve drug efficacy, a measure of the degree and consistency of symptom alleviation [37].

Testing relative efficacy (and potency) has proven to be a particularly contentious issue in the development of vanadium compounds as pharmaceutical agents [38–40]. It is generally conceded that the 'best' compound is the one, of a series, which is effective at the lowest dose, for the *same functional endpoints*. It is now abundantly obvious, however, that comparison between compounds, rated according to different measures of efficacy, does not tell the complete story. One compound may be more effective in terms of in vitro lipolysis inhibition, for instance, while another may be optimally effective when administered at a low dose orally for a prolonged period, and yet a third may show maximal potency when administered as an (one-time) oral gavage dose. Functional endpoints commonly chosen in diabetes drug candidates include glucose-lowering (from an initial hyperglycemic level), protein-tyrosine phosphatase (PTPase) inhibition, free fatty acid (FFA) release, and/or improved insulin sensitivity [16]. For anti-neoplastic and spermicidal potency, the relative efficacy may be strongly dependent on generation of heightened oxidative stress levels intracellularly, and the dosages required to

achieve these effects are of a different order of magnitude from those which elicit vanadium's anti-diabetic effects [41–43].

3. Vanadium-containing insulin enhancing agents

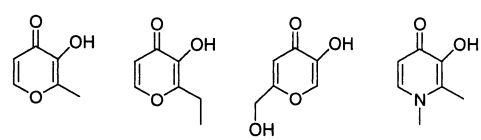
Many compounds have been proposed as 'insulin mimics.' This term may be somewhat misleading, in that vanadium can never entirely replicate insulin's plethora of effects [31]. Vanadium can mimic some, but not all, of insulin's actions and, most notably, does not function in this capacity in the complete absence of endogenous insulin. Thus, some investigators prefer to think in terms of 'insulin enhancement' for characterization of vanadium's anti-diabetic effects [44]. Herein we use these terms somewhat interchangeably, with this proviso in mind.

3.1. V(IV) insulin enhancing agents

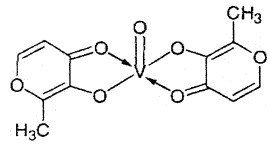
3.1.1. Pyronates and pyridinonates

As ligands for the design of vanadium complexes appropriate for use as insulin enhancing agents, 3-hydroxy-4-pyrones have proven to be exemplary. Maltol (Hma) and derivatives tend to possess moderate intrinsic bioactivity and a low toxicity profile [45]; thus, they are very good spectator ligands in biological applications [46]. Both maltol and ethyl maltol are by themselves approved food additives in many countries. In addition, Hma is well known for formation of stable, neutrally charged metal complexes which have an optimum combination of water-solubility, reasonable hydrolytic stability, and significant lipophilicity [23,46–48]. Pyrones and pyridinones can act as anionic chelating, bidentate O,O' ligands towards a number of biologically active metals [46–50].

Ligands structurally related to maltol include kojic acid (Hkoj), and Hdpp (1,2-dimethyl-3-hydroxy-4-pyridinone), both of which have substituents that can alter selectively the water-solubility, hydrolytic stability and lipophilicity of their metal complexes [49].



Bis(maltolato)oxovanadium(IV), BMOV or $VO(ma)_2$ (**1**), consists of vanadyl ($[VO^{2+}]$) bound to the anion of maltol (3-hydroxy-2-methyl-4-pyrone, Hma) [50]. Interest in maltol and close analogues, such as (the mis-named) ethylmaltol (3-hydroxy-2-ethyl-4-pyrone, Hema) and kojic acid (5-hydroxy-2-hydroxymethyl-4-pyrone, Hkoj), is partly due to their ability to deprotonate readily (pK_a values for Hma = 8.38, Hema = 8.78, Hkoj = 7.72) [51].



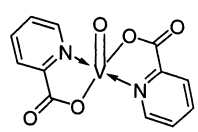
1, BMOV, $VO(ma)_2$

In the solid state, BMOV has a square pyramidal structure with the two maltolato ligands in a *trans* arrangement around the base of the square pyramid and the V=O unit axial. The V–(O)(hydroxo) and V–O(keto) distances (1.959(8) and 2.024(8) Å, respectively) were significantly different from one another and were much better resolved than their analogues in the other ma^- ligand [51].

BMOV is the most widely and intensively tested of the many proposed insulin mimetic vanadium complexes [38,39,50–55]. In addition to lowering glucose- and lipid-levels in vivo, BMOV delays or prevents long-term diabetes-induced pathology (including cardiomyopathy) and attenuates hyperinsulinemia and hyperlipidemia in genetically diabetic rats. The longest residence times for vanadium in vivo following oral administration of ^{48}V-BMOV were in bone (31 days), followed by liver (7 h) and kidney (4 h) [55]. On average in these three tissues, vanadium uptake is 2–3 times greater after oral ^{48}V-BMOV administration, compared to the same dose of $^{48}VOSO_4$. Bis(ethylmaltolato)oxovanadium(IV), BEOV, $VO(ema)_2$ [56], with slightly greater hydrolytic stability and lipophilicity, has longer turnover times in vivo, especially in bone and liver. Solubility decreases only slightly, and stability to oxidation remains unchanged from BMOV. BEOV successfully completed phase I clinical trials in early 2000.

3.1.2. Picolinates

Other compounds of the form $V^{IV}OL_2$, where L is a bidentate monoprotic ligand, have also proven effective in modifying insulin activity in vivo. One example is bis(pyridine-2-carboxylato)oxovanadium(IV), $VO(pic)_2$ (**2**), the synthesis of which was first reported in 1964 [57], but which was only recently characterized structurally (by analogy with the V(V) analogue) [58] and tested biologically [58,59].



2

Its V(V) analogue, the $[VO_2(pic)_2]^-$ anion, consists of a *cis*-VO_2^+ centre (V=O = 1.637(2), 1.632(2) Å, with an O=V=O angle of 104.3(1)°) and two picolinate ligands bound at an interplanar angle of 97.25° [58]. Extensive intramolecular hydrogen bonding was seen in $NH_4[VO_2(pic)_2]\cdot 2H_2O$, with one V=O hydrogen bonded to a water molecule. Bonding of a ligand hydrogen to an additional water molecule occurred through the electron deficient carboxylate *trans* to a V=O [58].

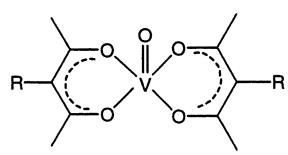
A methyl analogue of $VO(pic)_2$, bis(6-methylpyridine-2-carboxylato)oxovanadium(IV), VOMPA, has also been synthesized. It has been characterized biologically [60], potentiometrically [61], and by a new blood circulation monitoring-EPR method (BCM-ESR) [62] but, unfortunately, not structurally.

The tridentate chelating ligand pyridine-2,6-dicarboxylate (dipicolinate dianion, $dipic^{2-}$) can also chelate oxovanadium(IV) forming a six-coordinate complex, $VO[(dipic)(H_2O)_2]\cdot 2H_2O$, with a distorted octahedral coordination geometry around a central V(IV) atom [63]. A nitrogen donor atom was coordinated *trans* to the vanadyl oxygen and the V=O moiety had a shorter V–O distance (1.592(3) Å) than in $VO(pic)_2$ [63].

The insulin enhancing effects of picolinato chelates of oxovanadium(IV) have been clearly shown to be dependent on dose as well as delivery method. $VO(pic)_2$, 0.2 mmol kg^{-1} orally for 2 days, then 0.1 mmol kg^{-1} for 11 days, normalized plasma glucose in streptozotocin (STZ)-diabetic rats, a model of insulin-dependent diabetes (IDDM) [64]. Plasma insulin levels increased significantly during this trial [64]. By comparison, when $VO(pic)_2$ was given to STZ-diabetic rats as a 2.4 mM solution (drinking water substitute), the calculated dose averaging 1.0 mmol kg^{-1} d^{-1} was accompanied by consistent glucose-lowering and no insulin elevation, but with considerable evidence of gastrointestinal irritation [58]. Intraperitoneal (i.p.) administration of $VO(pic)_2$ at doses of 0.2, 0.1, and 0.06 mmol V kg^{-1} d^{-1} [58,64] also lowered plasma glucose levels, accompanied by increased bilirubin at the highest dose. In comparison to its methylpicolinate analogue, the picolinate complex had a less sustained response and was less effective as an inhibitor of FFA release in vitro; thus VOMPA was chosen for continued investigation [60,62,64]. On the other hand, comparing $VO(pic)_2$ with BMOV [58], the picolinate complex had lower solubility and more gastrointestinal irritation for an equivalent dose, suggesting that there is room for further structural improvement in order to increase bioavailability and lessen side effects.

3.1.3. Acetylacetonates

Synthesis and characterization of bis(2,4-pentanedionato-*O*,*O*)oxovanadium(IV), $VO(acac)_2$ (**3**), were first reported nearly a century ago [65]; however this, and several analogues have only recently been considered as potential insulin mimetic agents [39,66]. Both the 3-methyl- and 3-ethyl-2,4-pentanedionato vanadyl complexes, VO(Me-acac)$_2$ and VO(Et-acac)$_2$ (**4**), respectively, have been structurally characterized [66].



R=H, 3; CH_3; C_2H_5, 4

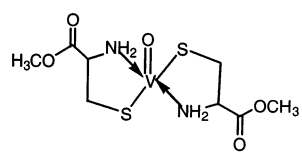
The vanadium atoms in these mononuclear complexes lie in distorted square pyramidal coordination environments. Both compounds have oxygen atoms coordi-

nating in the equatorial plane; apical coordination by the oxo group completes the square pyramidal geometry in each case. As with the parent compound $VO(acac)_2$, X-ray structure analysis shows that the vanadium atom is lying above the basal plane. The shorter V=O bond length for $VO(acac)_2$ (1.561(10) Å) compared with these in $VO(Me–acac)_2$ (1.592(2) Å) and $VO(Et–acac)_2$ (1.603(2) Å) suggests that Et–acac$^-$ and Me–acac$^-$ ligands have greater electron-donating abilities than acac$^-$. These distances are all much shorter than the V=O length in $VO(ma)_2$ (also a $VO(O_4)$ coordination sphere, vide supra).

In an in vitro study, $VO(acac)_2$, 5–100 μM, was more effective than vanadyl sulphate (VS) in stimulating lipogenesis in isolated fat cells, and had identical effectiveness in stimulating activity of a cytosolic protein kinase (CytPTK) [67]. Intraperitoneal injection (25 μmol kg^{-1}) of $VO(acac)_2$ lowered slightly plasma glucose levels in STZ-diabetic rats, though not to normal glucose levels; $VO(Etacac)_2$ at the same dose was ineffective [39]. BMOV, $VO(acac)_2$, and $VO(Etacac)_2$, 0.4 mM orally, were equally (mildly) effective in glucose-lowering when given in the drinking water over an 8-week treatment period, but were significantly different from VS at the same dose. In fact, the only clearly relevant physiological difference in this comparative group was between the ratio of vanadium intake/plasma vanadium levels, with VS having a remarkably higher ratio compared to all oxovanadium(IV) complexes tested [39].

3.1.4. Dicarboxylate ester–oxovanadium(IV) complexes

Originally investigated as models for the interaction of vanadium(IV) with bioligands, complexes of oxovanadium(IV) with a series of dicarboxylate ligands (oxalate, glutarate, succinate, malonate) proved effective orally as insulin mimetic agents [68]. Only the cysteine methyl ester–oxovanadium(IV) complex, VCys (**5**), was characterized by X-ray structure analysis [69].



5

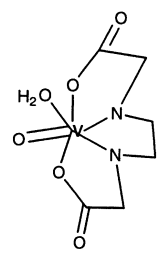
The geometry around the oxovanadium(IV) in VCys appeared to be penta-coordinate around the central vanadium atom, square pyramidal with two-fold symmetry and each pair of nitrogen and sulphur atoms mutually *trans*. Bond distances V–S (2.322 Å) and V=O (1.616 Å) were comparable to those in other vanadyl–dithiolate complexes. The V–N bond distance (2.132 Å) was short compared with other oxovanadium(IV) complexes. The bond angles O=V–N and O=V–S of 98.06 and 114.13°, respectively, suggest the expected out-of-plane placement for the vanadium atom.

At the doses tested for bioactivity (0.06 and 0.20 mmol V kg^{-1}), VCys and the bis ligand analogues of malonate, tartarate, and salicylaldehyde were indistinguishable from one another in terms of glucose-lowering ability in STZ-diabetic rats. The higher dose appeared to be significantly more effective than the lower dose in all

cases. An oxalic acid analogue, bis(oxalato)oxovanadate(IV) dianion, was less effective than the others; significant glucose-lowering was seen only at the 0.20 mmol V kg^{-1} dose [68].

3.1.5. Peptide bound complexes

Replacement of two bidentate ligands with one tetradentate ligand can be expected to increase stability constants of resulting complexes via the chelate effect [70]. Hydrated oxovanadium(IV) complexes of the *N*-alkylated derivatives of glycine and methionine (VO(XeX)(H_2O)], where XeX = *N*,*N*′-ethylene bis-α-amino acid, have been synthesized and characterized [71]. Blue [VO(GeG)(H_2O)] (**6**), GeG = *N*,*N*′-ethylene bis-glycine, a.k.a. EDDA, has a distorted octahedral coordination. The V=O bond length (1.595(1) Å) is close to that in $VO(acac)_2$, and the V–N bond lengths are similar to those determined for $VO(pic)_2$ (vide supra), with V–N = 2.330(1), 2.106(1) Å. A novel, S-containing tetradentate complex of this type, [VO(MeM)(H_2O)], MeM = *N*,*N*′-ethylene bis-(*S*)-methionine, was also characterized [71]. The distorted octahedral structure has a V(IV) atom in the equatorial plane, with a mean deviation of 0.351 Å, with one N and one O atom apical, and V=O somewhat lengthened compared to that in [VO(GeG)(H_2O)] (1.602(2) Å). The V–N bonds are also somewhat lengthened in [VO(MeM)(H_2O)]: V–N = 2.370(2) and 2.140(2) Å [71].



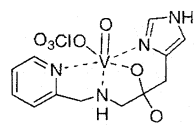
6

Insulin mimetic activity of these two complexes, compared against vanadyl sulphate (VS), was evaluated only in vitro, by determination of free fatty acid release (FFA) from fat cells, a common assay which measures the degree of inhibition of lipolysis [71]. At the concentrations tested (0.1, 0.5, and 1 mM), VS was not significantly inhibitory, and [VO(MeM)(H_2O)] was only slightly inhibitory (compared to control). In contrast, [VO(GeG)(H_2O)] did inhibit FFA release substantially, in a dose dependent fashion, within the concentration range tested.

An extension of this study varied the amino acids (and derivatives) incorporated in XeX ligands, including methylglycine, L- and D-alanine, L- and D-valine, L- and D-methionine, and L- and D-proline [72]. Effects of chirality on IC_{50} (vanadium complex concentration required to inhibit FFA release by 50%) were compared with the influence of partition coefficients in *n*-octanol/saline systems, the pK_a of the amino acids in the ligands, and the redox potentials of the complexes (with respect to Ag | AgCl, determined electrochemically in 0.1 M phosphate buffer, pH 7.0) on this same measure of insulin enhancement [72]. Chiral amino acid-contain-

ing oxovanadium(IV) complexes of the Δ-type configuration tended to inhibit FFA release at lower concentrations when the complexes were more highly lipophilic and had lower redox potentials or, within $9.0 \leq pK_a \leq 10.4$, when they had a lower pK_a. Achiral complexes showed no such dependence on lipophilicity, redox potential or ease of deprotonation of the amino acid within the ligand.

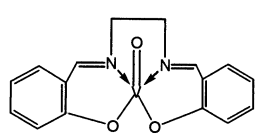
A pseudo-tripod-type tetradentate variant on this theme, *N*,*N*′-ethylene-(*S*)-histidine-(*S*)-tyrosine-oxovanadium(IV), [VO(HeY)], has been synthesized using a one pot synthesis method to assemble the ligand at V [73]. The V(IV) ion in this complex lay in an equatorial least-squares plane, with a mean deviation of 0.001 Å. V=O and V–N bond lengths were closely similar to those of [VO(MeM)(H_2O)], V=O = 1.603(3) Å; V–N = 2.324(3), 2.127(3) Å, but with a third shorter V–N bond (2.111(2) Å). Using the same concentration ranges as above, this complex did not inhibit FFA release, but a related compound (not characterized by X-ray crystallography) *N*-2-pyridylmethyl-(*S*)-histidine methyl oxovanadium(IV)perchlorate, [VO(^{pm}H)(ClO_4)] (**7**), at 1 mM inhibited at the same concentration as did [VO(GeG)(H_2O)], but was ineffective at lower concentrations.



7

3.1.6. V(IV) SALEN derivatives

Various vanadium complexes of the dianionic tetradentate Schiff base ligand salicylideneimine (SALEN) have been proposed for potential use as insulin mimetic agents [70]. A complete overview of the coordination chemistry of such complexes has appeared recently [70]. These ligands are of particular interest because they provide coordination environments which efficiently stabilize different oxidation states of vanadium, while still providing active sites capable of binding other molecules. Dimensions around the V atom tend to be short (V=O = 1.589(4) Å, V–O = 1.919(4), 1.917(4) Å) for the V(IV) complexes, which had the expected five-coordinate square pyramidal geometry. For a bidentate Schiff base V(III) complex, tris(2-phenolato-*N*-(*n*-propyl)acetophenoneimine)vanadium(III), whose X-ray structure was described [74], the geometry was six coordinate octahedral with atomic distances in line with similar complexes.



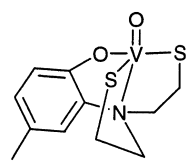
8

To date, only VO(SALEN) (**8**) has been tested for insulin mimetic activity from among these complexes [75]. Unlike most biological testing for new anti-diabetic

agents, this was carried out in alloxan-diabetic rats, in which blood glucose levels decreased from hyperglycemic to hypoglycemic during oral intubation (VO(SALEN), 0.15 mmol V kg^{-1} d^{-1} for 30 days). Withdrawal of treatment brought an immediate reversion to hyperglycemia. Blood glucose levels were reduced over the treatment period; liver hexokinase activity was restored, and other carbohydrate metabolism enzymes in liver and kidney were normalized. Risk of hypoglycemia, however, presents a serious drawback to potential use as an insulin mimetic.

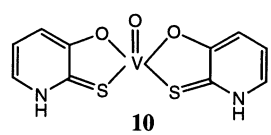
3.1.7. Sulphur-containing ligands

Higher oxidation states of vanadium are known to be relatively hard acids, therefore preferring to bind with nitrogen or oxygen over sulphur atoms. Thiolate vanadium complexes are then easier to reduce and harder to oxidize than their nitrogen- or oxygen-coordinating analogues. V–S bonds average 2.35 Å; V=O bonds average 1.62 Å. In the vanadium thiolate complexes which have been structurally described so far, a slight lengthening of the V=O bond, relative to other penta-coordinate oxovanadium complexes, has been ascribed to π-interactions between the sulphur lone pairs and the metal d-orbitals, with increased delocalization of the vanadium(V) d-electron [5]. VCys (vide supra), an oxovanadium(IV) complex tested early for insulin enhancing potential [68], is an example of such a compound.



9

The trigonal bipyramidal V(V) complex, 2-bis(ethylsulphenato)amino-4-methylphenolatooxovanadium(V) (**9**), has an oxo ligand as one of the axial ligands of the molecule [76]. The two thiolate sulphur atoms and one phenolate oxygen make up the equatorial plane; coordination of the amine nitrogen to the oxo moiety is *trans*. This η^2,η^2-disulphenate complex, with a V(V) oxidation state, has O, S, and N coordination and, as a structural analogue of active site thiols in protein-tyrosine phosphatase (PTPase) enzymes, is a good candidate for insulin mimesis, but has as yet not been tested [76].



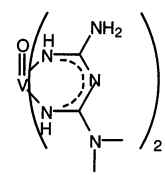
10

Bis(2-mercapto-3-pyridinol)oxovanadium(IV) (**10**) [77], and the closely related bis(2-mercaptopyridine-*N*-oxide)oxovanadium(IV) [78] both appear to have potential as insulin mimetic agents, though neither has yet been tested for bioactivity. The incompletely characterized bis(pyrrolidine-*N*-carbodithioato)oxovanadium(IV) [37] and analogues [40], although they did lower plasma glucose and inhibit free

fatty acid release, also stimulated increased bilirubin production at therapeutically relevant doses [40]. The more recently reported (but also not yet structurally characterized), bis(1-oxy-2-pyridinethiolato)oxovanadium(IV) [79] appears to be less prone to this adverse effect.

3.1.8. Hypoglycemic agents as ligands

Oral hypoglycemic agents currently on the market for drug use may also have potential as ligands for vanadium complexation [80]. A synergistic, or at least additive, effect would be a highly desirable outcome of this approach, allowing minimization of any negative side effects of either the oral hypoglycemic or the vanadium moiety. In a series of vanadyl biguanides [80], including vanadyl metformin, $VO(metf)_2 \cdot H_2O$ (**11**), no synergy was observed. The compounds tested (at 0.12 mmol kg^{-1} i.p. and 0.60 mmol kg^{-1} p.o.) were almost as effective as BMOV and BEOV; however, the vanadium dose required for glucose-lowering was approximately the same, and the glucose-lowering effect was not as sustained with vanadyl metformin as it is with BMOV or BEOV [80].

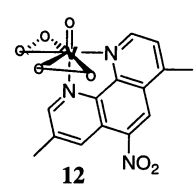


11

Other $VO(bg)_2$ complexes, where bg is a biguanide [80], as well as $VO(oz)_2$ and $VO(thoz)_2$, where oz is the anion of 2-(2′-hydroxyphenyl)-2-oxazoline, and thoz is the 2-thiazoline derivative thereof [58], and some of the possible $VO(Rpyr)_2$, where Rpyr is an N-functionalized 3-hydroxy-4-pyridinone (unpublished work), have been synthesized and characterized (some structurally by X-ray crystallography). Low aqueous solubility deterred further investigation of these compounds as insulin enhancing agents.

3.2. V(V) protein-tyrosine phosphatase inhibitors

3.2.1. Mono- and di-peroxovanadates



12

Bidentate ligands, such as 1,10 phenanthroline, can stabilize oxoperoxovanadate(V) compounds of the general type $[VO(O_2)_2(L)]^-$, as NH_4^+ or K^+ salts. X-ray structure analysis of potassium (5-nitro-1,10-phenanthroline-N^1,N^{10})oxodiperoxo-

vanadate(V) dihydrate (**12**) [81] shows its pentagonal bipyramidal geometry, with the pentagonal plane consisting of two slightly asymmetric peroxo groups and one N-atom from the phenanthroline ligand. The V–O bonds were slightly shorter in one peroxo ligand than in the other (average: 1.906(6) vs. 1.878(6) Å).

In tripotassium oxodiperoxo(2,4-pyridinedicarboxylato)vanadate(V) [82], the structure approximates a distorted trigonal bipyramid (if one assigns one coordination site to each peroxo ligand) with the inner coordination sphere of the vanadium atom consisting of one nitrogen and six oxygen atoms. The closely related peroxopicolinato complex, $K_3[VO(O_2)_2(OHpic)]\cdot 3H_2O$, more closely approximates a distorted pentagonal bipyramid geometry, with the oxo ligand and the carboxylato ligands in the axial positions. Peroxovanadates (pV) of this type are generally much more efficient than vanadate at oxidative coupling of bio-molecules such as cysteine and citrate [83,84].

In dipotassium (3-hydroxypyridine-2-carboxylato)(oxo)diperoxovanadate(V), coordination is via the lone nitrogen atom and one of the carboxylate oxygens. Here again, the geometry around the vanadium atom is a distorted pentagonal bipyramid [83].

3.2.2. Peroxo-complexes with amino acids and analogues

Well before the 1990s interest in peroxo-vanadium compounds as possible insulin mimics, peroxovanadium(V) complexes with polycarboxylato and amino polycarboxylato heteroligands were synthesized as potential model systems for interaction of vanadium in various biological milieux [33,85,86]. More recently, a series of oxodiperoxovanadate complexes containing coordinated amino acids, of the general type $K_n[VO(O_2)_2AA]\cdot 2H_2O$, where AA = l-asparagine, l-phenylglycine, d,l-homocystine, have been synthesized, partially characterized, and proposed (but not tested) as suitable insulin mimetic agents [87]. All were highly soluble in water, but also hygroscopic, and light and temperature sensitive, obviating much diabetes therapy interest.

Histidine residues coordinated to vanadium atoms are central to the function of the vanadium-containing haloperoxidases and some phosphorylases. A model of this interaction, an imidazole peroxovanadium complex, has been synthesized and characterized [88], demonstrating that V(V) in a peroxo compound can be six-coordinate. Here the coordination environment of the vanadium atom was close to a pentagonal pyramid, with the oxo group occupying the axial position, and the base formed by an imidazole nitrogen and two peroxo groups. Imidazolium imidazoleoxodiperoxovanadate(V), was effective in vitro (1 μM V) at increasing insulin receptor phosphorylation up to four times greater than maximal insulin stimulation, and in increasing glucose uptake substantially (in rat adipocytes and epitrochlearis muscle) in the presence of submaximal insulin [88].

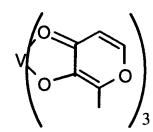
Since hydroxylamine is isoelectronic with hydrogen peroxide, vanadium(V) complexes of hydroxylamine analogues [89–91] might be expected to exhibit some structural similarities to peroxovanadates. Nonetheless, hydroxylamine vanadyl complexes appear to be much weaker oxidants of thiol groups than peroxovanadates and, although they are also potent inhibitors of some PTPases, the mecha-

nisms for the two types of complexes appear to differ. If increased oxidative stress is a desirable feature of vanadium inhibition (vide infra), then hydroxylamine complexes can be expected to be less effective [92].

3.3. V(III) complexes

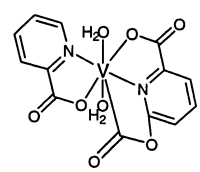
Mononuclear coordination complexes of V(III) are generally paramagnetic (two unpaired electrons), six-coordinate, hydrolytically stable, axially symmetric, having an octahedral or pseudo-octahedral geometry [5]. They are not commonly considered for therapeutic applications due to their known rapid oxidation to V(IV/V) in aqueous solutions of pH > 3 [93,94].

The first synthesis and characterization of complexes of V(III) with maltol (and analogues) as candidate anti-diabetic agents was accomplished only very recently [95]. As with other metal chelates of these [96–98] and related ligands [99], reasonable hydrolytic and thermodynamic stabilities were anticipated; the air stability of $V(ma)_3$ was an unexpected advantage. Treatment with either $V(ma)_3$ or $VO(ma)_2$ resulted in glucose-lowering in STZ-diabetic rats of comparable and significant magnitudes, both when administered by i.p. injection and orally, with no overt toxicity other than gastrointestinal distress, and no fatalities [95].



13

Neither $V(koj)_3 \cdot H_2O$ nor $V(dpp)_3 \cdot 12H_2O$ proved active as insulin enhancing agents; however $V(dpp)_3 \cdot 12H_2O$ (**13**) was characterized structurally by X-ray crystallography [95]. In $V(dpp)_3 \cdot 12H_2O$, there was a compression of the octahedron along the trigonal axis, as evinced by a large exocyclic O–V–O angle of 98° as well as in a relatively small O–V–O bite angle of 81°.



14

Diaquadipicolinatopicolinatovanadium(III) (**14**) has the unusual feature of being seven coordinate V(III) with a pentagonal bipyramidal structure [100]. The monomeric complex contains two different α-*N*-heterocyclic carboxylate donors around a V(III) acceptor centre. The dipicolinate dianion, with its two carboxylate groups in *ortho* positions with respect to the pyridine nitrogen, is potentially tridentate. The ligand has three protonation states, DPA^{2-}, $DPAH^{-}$, or $DPAH_2$

with acidity constants $pK_{a1} = 2.10$ and $pK_{a2} = 4.68$ [100]. In this heterocyclic V(III) complex, the geometry around the V(III) centre is a slightly distorted pentagonal bipyramid with dipicolinate and picolinate ligands occupying the pentagonal equatorial plane and two coordinated water molecules in the two axial sites. The bite angles around the vanadium atom range from 71 to 73° summing the in-plane angles to 360.3°, illustrating the high planarity of the pentagon. The bond angle involving the axial water oxygen atoms and the V atom is 177°, completing a symmetric pentagonal bipyramid structure [100]. This complex has not been tested for biological activity; however, based on the insulin enhancing capability of its oxovanadium(IV) analogue ($VO(pic)_2$), there is every reason to anticipate that the V(III) picolinate complex might also be pharmacologically interesting.

4. Other vanadium-based therapeutic agents

4.1. Anti-neoplastic agents and chemotherapeutic complexes

Besides insulin enhancing effects, other pharmacological activities of vanadium compounds include inhibiting tumour growth and prophylaxis against carcinogenesis. Not only is redox state important, but the particular organic ligand which coordinates the V atom also influences the anti-neoplastic potential of individual vanadium(V) complexes [42,101,102]. In a comparison of 15 oxovanadium(IV) complexes examined for their cytotoxic potential against 14 distinct human cancer cell lines, the most important feature of the ancillary ligand for maximal potency was dimethyl substitution on phenanthroline rings, with bis-chelated phen compounds more active than mono-chelated phen compounds [102].

Based on their relative abilities to inhibit phosphotyrosine phosphatases, potassium salts of peroxovanadium(V) bipyridine, $[VO(O_2)_2bipy]^-$, $[VO(O)_2pic]^{2-}$ (bpV(pic)), and $[VO(O_2)_2(HOpic)]^{2-}$ (bpV(HOpic), would be expected to be particularly effective as cytotoxic agents [103]. Peroxovanadate(V) complexes have long been known for their anti-tumour activity (in this case, against L1210 murine leukemia) [104], with the ammonium salt of peroxovanadate(V) iminodiacetate showing remarkable resistance to decomposition, always an overriding concern with peroxovanadates [104,105]. An X-ray structure analysis of $NH_4[VO(O_2)IDA]$ indicated that this peroxo heteroligand complex was polymeric in the solid state [104].

Organometallic complexes of vanadium(IV) with cyclopentadienyl ($C_5H_5^- = Cp^-$) moieties, vanadocenes (VCp_2), exhibit anti-tumour and anti-proliferative properties both in vitro and in vivo, primarily via oxidative damage [106]. Of these, a recent comparison test in the human testicular cancer cell lines, Tera-2 and Ntera-2, using both 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays and apoptosis assays, led to the conclusion that the $VCp_2(NCSe)_2$ was most potent [101].

Amino acid complexes of vanadium have also been proposed as anti-tumour agents [107]; a recently prepared V(III)–cysteine complex showed nearly additive effects, compared with either VS or cysteine alone, against benzo(α)pyrene-induced Leiomyosarcomas in Wistar rats [108].

4.2. Vanadium compounds as spermicides and anti-inflammatories

Vanadocene complexes have also shown considerable potential as sperm-immobilizing agents [41,107]. A vanadocene(IV) complex of diethyldithiocarbamate, $[VCp_2(Et_2dtc)]BF_4$, was characterized by X-ray structure analysis; the coordination geometry around the central vanadium atom was roughly tetrahedral, with two planar cyclopentadienyl rings and two dithiocarbamate S atoms coordinating V(IV) [41].

Exposure of motile sperm to this and related vanadocene complexes (0.12–125 μM) resulted in sperm immobilization with $EC_{50} = 1.1$ μM for $[VCp_2(Et_2dtc)]BF_4$ [41]. Other mono- and bis-ligated oxovanadium(IV) cationic complexes, with ligands such as phenanthrolines, bipyridines, and acetophenones, had EC_{50} values ranging 5.5–118 μM [109], with the most active being (aqua)bis(5-chloro-1,10-phenanthroline)oxovanadium(IV) sulphate, $[VO(Cl–phen)_2(H_2O)]SO_4$, and the least active, (diaqua)(2,2′-bipyridyl)oxovanadium(IV) sulphate, $[VO(bipy)(H_2O)_2]SO_4$. A neutral functionalized acetophenone complex, bis(5′-bromo-2′-hydroxyacetophenone)oxovanadium(IV)sulphate, $VO(Br, OH–acph)_2$, displayed intermediate activity in sperm immobilization, but was exceptionally rapid ($T_{1/2} = 38$ s) [109]. More recently, dichlorobis(pentamethylcyclopentadienyl)vanadium(IV) was shown to induce sperm immobilization ($T_{1/2} < 15$ s) using a boar sperm model system which has been proposed as a useful screen [110]. Because human sperm are extremely sensitive to oxidative stress, the generation of reactive oxygen species by even very short exposure to vanadocenes may result in spermidical and apoptosis-inducing properties [109,111].

Activation of the nuclear transcription factor, NF-κB, is required for expression of various inducible target genes related to immune and inflammatory responses [112]. That some oxovanadium complexes may have differential effects with regard to NF-κB activation has been explored [53,60,112,113], with BMOV (compared with pV(phen) and Na_3VO_4) showing evidence of NF-κB activation without accompanying oxidative stress [53]. Use of various vanadium-containing phosphotyrosine phosphatase inhibitors as regulators of cell proliferation [114,115], including as a method of treating arthropies such as arthritis, has been patented [116] or published [117]. Nonetheless, to our knowledge, there have been no published reports of experimental trials of this treatment method in the scientific literature.

5. Putative mechanism(s) of vanadium's effects

5.1. Advantages and disadvantages of oxidative stress

Increased production of reactive oxygen species in diabetes is a contributor to secondary complications; e.g. microangiopathy, atherosclerosis, renal failure, neuropathy, which are major causes of morbidity and mortality in this metabolic disorder [118,119]. Therapeutic agents that would tend to exacerbate an already heightened oxidative stress are thus unlikely to be viable candidate compounds for

chronic use in diabetes [53]. On the other hand, for anti-tumour potential, increased oxidative stress could well have a positive effect; tumour cells are particularly susceptible to oxidative stress [120]. Comparison of sodium orthovanadate, vanadyl sulphate, BMOV and the V(V) analogue of BMOV, *cis*-bis(maltolato)dioxovanadate(V) anion (BMV), suggested that BMV was the least cytotoxic and the weakest inducer of morphological changes. All promoted phosphorylation of tyrosine in several proteins, an effect that was more pronounced at low (10 μM) doses than at higher doses (25 or 50 μM). Nonetheless, BMOV PTPase inhibition was independent of oxidative stress production within lymphocytes, whereas sodium oxodiperoxo(1,10-phenanthroline)vanadate(V) required increased intracellular oxidative stress for activation of NF-κB and consequent PTPase inhibition [53].

The favoured underlying mechanism of peroxovanadium compounds as anti-neoplastic agents is increased oxidative stress [108,114,115]. Peroxovanadium complexes differ from vanadates in being stronger oxidizing agents than glutathione [42,121]. The most immediate observed effect of relevance to the anti-tumour potential of peroxovanadium complexes is DNA cleavage at low μM intracellular V concentrations in vitro [122]. This redox capability is also important for use of vanadium compounds as spermicides [110].

5.2. Other considerations of mechanism

The pleiotrophic effects of vanadium in vivo include numerous enzymatic effects beyond inhibition of phosphotyrosine phosphatases and stimulation of tyrosine kinases [121,123]. Peroxovanadates appear to facilitate their insulin-like effects exclusively through receptor activation and IRS-1 phosphorylating pathways, unlike oxovanadium(IV) complexes [31]. Stimulation of glucose transport and GLUT translocation in response to oral administration of vanadium compounds mimic insulin in direction; however, the mechanisms appear to be dissimilar [123]. Modulation of intracellular signaling may be mediated through sustained or oscillatory Ca^{2+} ion regulation, induced by supraphysiological levels of vanadium intracellularly [124].

6. Summary and conclusions

Vanadium compounds are being developed for pharmaceutical use as insulin enhancing agents in both insulin dependent and non-insulin dependent diabetes, as anti-neoplastic agents, as spermicides, and for a variety of other uses as well. Design of new vanadium-containing coordination complexes for these various applications requires attention to the redox activity of vanadium, which can be either beneficial or deleterious, depending on the intended use. The stereochemical flexibility of oxovanadium(IV) and (V) compounds, and the tendency of V(III) compounds to oxidize readily to V(IV) and (V) are unique features of vanadium which can be applied to specific and innovative metallopharmaceutical design. Some oxovanadiu-

m(IV) complexes are now in clinical trials, and with all of the potential uses being developed, we can expect to see many more in future.

Acknowledgements

Work in the authors' laboratories is currently supported by the Natural Sciences and Engineering Research Council (NSERC) of Canada, Kinetek Pharmaceuticals, and the Medical Research Council (MRC) of Canada. C.O. acknowledges the Alexander von Humboldt Foundation for a Research Award and Professor Dr F.E. Hahn for his hospitality in 1999–2000.

References

[1] A. Butler, The coordination and redox chemistry of vanadium in aqueous solution, in: N.D. Chasteen (Ed.), Vanadium in Biological Systems, Kluwer, Dordrecht, 1990 (chap. 2).
[2] D.W. Boyd, K. Kustin, Adv. Inorg. Biochem. 6 (1984) 311.
[3] K.H. Thompson, J.H. McNeill, C. Orvig, Top. Biol. Inorg. Chem. 2 (1999) 139.
[4] A. Butler, C.J. Carrano, Coord. Chem. Rev. 109 (1991) 61.
[5] E.M. Page, S.A. Wass, Coord. Chem. Rev. 164 (1997) 203.
[6] E.M. Page, Coord. Chem. Rev. 172 (1998) 111.
[7] D. Rehder, Coord. Chem. Rev. 182 (1999) 297.
[8] K.H. Thompson, BioFactors 10 (1999) 43.
[9] N. Sekar, J. Li, Y. Shechter, Crit. Rev. Biochem. Mol. Biol. 31 (1996) 339.
[10] F.H. Nielsen, Metal Ions Biol. Syst. 31 (1995) 543.
[11] K.H. Thompson, M. Battel, J.H. McNeill, Toxicology of vanadium in mammals, in: J.O. Nriagu (Ed.), Vanadium in the Environment. Part 2: Health Effects, Wiley, New York, 1998 (chap. 2).
[12] N.D. Chasteen, Vanadium in Biological Systems: Physiology and Biology, Kluwer Academic, Dordrecht, 1990.
[13] H. Sigel, A. Sigel (Eds.), Metal Ions in Biological Systems, vol. 31, Marcel Dekker, New York, 1995.
[14] A.S. Tracey, D.C. Crans (Eds.), Vanadium Compounds. Chemistry, Biochemistry and Therapeutic Applications. In: ACS Symposium Series, vol. 711, American Chemical Society, Washington, DC, 1998.
[15] J.O Nriagu (Ed.), Vanadium in the Environment. Part 1: Chemistry and Biochemistry, Wiley, New York, 1998.
[16] K.H. Thompson, J.H. McNeill, C. Orvig, Chem. Rev. 99 (1999) 2561.
[17] R.B. Rucker, B. Lönnerdal, C.L. Keen, Intestinal absorption of nutritionally important trace elements, in: L.R. Johnson (Ed.), Physiology of the Gastrointestinal Tract, 3rd ed., Raven Press, New York, 1994 (chap. 66).
[18] F.H. Nielsen, Vanadium, in: B.L. O'Dell, R.A. Sunde (Eds.), Handbook of Nutritionally Essential Mineral Elements, Marcel Dekker, New York, 1997 (chap. 22).
[19] C.W. Schwietert, J.P. McCue, Coord. Chem. Rev. 184 (1999) 67.
[20] M.J. Heeg, S.S. Jurisson, Acc. Chem. Res. 32 (1999) 1053.
[21] Z. Guo, P.J. Sadler, Angew. Chem. Int. Ed. Engl. 38 (1999) 1512.
[22] A.M.T. Bygott, A.M. Sargeson, Inorg. Chem. 37 (1998) 4795.
[23] P. Comba, Coord. Chem. Rev. 123 (1993) 1.
[24] M. Weber, D. Kuppert, K. Hegetschweiler, V. Gramlich, Inorg. Chem. 38 (1999) 859.
[25] B.L. Rai, Z.D. Liu, D.Y. Liu, S.L. Lu, R.C. Hider, Eur. J. Med. Chem. 34 (1999) 475.
[26] W.R. Harris, B. Yang, S. Abdollahi, Y. Hamada, J. Inorg. Biochem. 76 (1999) 231.

[27] M. Zhang, M. Zhou, R.L. Van Etten, C.V. Stauffacher, Biochemistry 36 (1997) 15.
[28] D.C. Crans, S.S. Amin, A.D. Keramidas, Chemistry of relevance to vanadium in the environment, in: J.O. Nriagu (Ed.), Vanadium in the Environment. Part 1: Chemistry and Biochemistry, Wiley, New York, 1988 (chap. 4).
[29] G.R. Willsky, Vanadium in the biosphere, in: D. Chasteen (Ed.), Vanadium in Biological Systems, Kluwer, Dordrecht, 1990 (chap. 1).
[30] A. Butler, J. Walker, Chem. Rev. 93 (1993) 1937.
[31] G. Elberg, J. Li, Y. Shechter, Mechanisms of actions of vanadium in mediating the biological effects of insulin, in: J.O. Nriagu (Ed.), Vanadium in the Environment. Part 2: Health Effects, Wiley, New York, 1998 (chap. 14).
[32] P.J. Stankiewicz, A.S. Tracey, Metal Ions Biol. Syst. 31 (1995) 249.
[33] C. Djordjevic, B.C. Puryear, N. Vletic, C.J. Abelt, S.J. Sheffield, Inorg. Chem. 27 (1988) 2926.
[34] W. Mertz, C.O. Abernathy, S.S. Olin (Eds.), Risk Assessment of Essential Elements, ILSI Press, Washington, DC, 1994.
[35] G.W. Evans, D.J. Pouchnik, J. Inorg. Biochem. 49 (1993) 177.
[36] T.W. Kensler, J.D. Groopman, T.R. Sutter, T.J. Curphey, B.D. Roebuck, Chem. Res. Toxicol. 12 (1999) 113.
[37] H. Watanabe, M. Nakai, K. Komazawa, H. Sakurai, J. Med. Chem. 37 (1994) 876.
[38] K.H. Thompson, V.G. Yuen, J.H. McNeill, C. Orvig, in: A.S. Tracey, D.C. Crans (Eds.), Vanadium Compounds. Chemistry, Biochemistry and Therapeutic Applications. In: ACS Symposium Series, vol. 711, American Chemical Society, Washington, DC, 1998, p. 329 (chap. 26).
[39] B.A. Reul, S.S. Amin, J-P. Buchet, L.N. Ongemba, D.C. Crans, S.M. Brichard, Br. J. Pharmacol. 126 (1999) 467.
[40] H. Sakurai, H. Watanabe, H. Tamura, H. Yasui, R. Matsushita, J. Takada, Inorg. Chim. Acta 283 (1998) 175.
[41] P. Ghosh, S. Ghosh, O.J. D'Cruz, F.M. Uckun, J. Inorg. Biochem. 72 (1998) 89.
[42] C. Cuncic, N. Detich, D. Ethier, A.S. Tracey, M.J. Gresser, C. Ramachandran, J. Biol. Inorg. Chem. 4 (1999) 354.
[43] V.C. Sálice, A.M. Cortizo, C.L. Gómez Dumm, S.B. Etcheverry, Mol. Cell. Biochem. 198 (1999) 119.
[44] M.C. Cam, R.W. Brownsey, J.H. McNeill, Can. J. Physiol. Pharmacol. 78 (2001) 829.
[45] M.A. Barrand, B.A. Callingham, R.C. Hider, J. Pharm. Pharmacol. 39 (1987) 203.
[46] D.J. Clevette, W.O. Nelson, A. Nordin, C. Orvig, S. Sjöberg, Inorg. Chem. 28 (1989) 2079.
[47] M.M. Finnegan, S.J. Rettig, C. Orvig, J. Am. Chem. Soc. 108 (1986) 5033.
[48] S.J. Lord, N.A. Epstein, R.L. Paddock, C.M. Vogels, T.L. Hennigar, M.J. Zaworotko, et al., Can. J. Chem. 77 (1999) 1249.
[49] W.O. Nelson, T.B. Karpishin, S.J. Rettig, C. Orvig, Can. J. Chem. 66 (1988) 123.
[50] J.H. McNeill, V.G. Yuen, H.R. Hoveyda, C. Orvig, J. Med. Chem. 35 (1992) 1489.
[51] P. Caravan, L. Gelmini, N. Glover, F.G. Herring, H. Li, J.H. McNeill, et al., J. Am. Chem. Soc. 117 (1995) 12759.
[52] J. Burgess, B. De Castro, C. Oliveira, M. Rangal, W. Schlindwein, Polyhedron 16 (1997) 789.
[53] C.M. Krejsa, S.G. Nadler, J.M. Esselstyn, T.J. Kavanagh, J.A. Ledbetter, G.L. Schieven, J. Biol. Chem. 272 (1997) 11541.
[54] V.G. Yuen, P. Caravan, L. Gelmini, N. Glover, J.H. McNeill, I.A. Setyawati, et al., J. Inorg. Biochem. 68 (1997) 109.
[55] I.A. Setyawati, K.H. Thompson, V.G. Yuen, T. Sun, M. Battell, D.M. Lyster, et al., J. Appl. Physiol. 84 (1998) 569.
[56] S.A. Dikanov, B.D. Liboiron, K.H. Thompson, E. Vera, V.G. Yuen, J.H. McNeill, et al., J. Am. Chem. Soc. 121 (1999) 11004.
[57] R.L. Dhutta, S. Ghosh, S. Lahiry, Sci. Cult. 30 (1964) 551.
[58] M. Melchior, K.H. Thompson, J.M. Jong, S.J. Rettig, E. Shuter, V.G. Yuen, et al., Inorg. Chem. 38 (1999) 2288.
[59] H. Sakurai, K. Fujii, H. Watanabe, H. Tamura, Biochem. Biophys. Res. Commun. 214 (1995) 1095.

[60] Y. Fujisawa, H. Sakurai, Chem. Pharm. Bull. 47 (1999) 1668.
[61] E. Kiss, E. Garribba, G. Micera, T. Kiss, H. Sakurai, J. Inorg. Biochem. 78 (2000) 97.
[62] H. Yasui, K. Takechi, H. Sakurai, J. Inorg. Biochem. 78 (2000) 185.
[63] M. Chatterjee, S. Ghosh, A.K. Nandi, Transition Met. Chem. 24 (1999) 183.
[64] S. Fujimoto, K. Fujii, H. Yasui, R. Matsushita, J. Takada, H. Sakurai, J. Clin. Biochem. Nutr. 23 (1997) 113.
[65] G.T. Morgan, H.W. Moss, J. Chem. Soc. 103 (1914) 78.
[66] S.S. Amin, K. Cryer, B. Zhang, S.K. Dutta, S.S. Eaton, O.P. Anderson, et al., Inorg. Chem. 39 (2000) 406.
[67] J. Li, G. Elberg, D.C. Crans, Y. Shechter, Biochemistry 35 (1996) 8314.
[68] H. Sakurai, K. Tsuchiya, M. Nukatsuka, J. Kawada, S. Ishikawa, H. Yoshida, et al., J. Clin. Biochem. Nutr. 8 (1990) 193.
[69] H. Sakurai, Z-I. Taira, N. Sakai, Inorg. Chim. Acta 151 (1988) 85.
[70] M.M. Aly, J. Coord. Chem. 43 (1998) 89.
[71] K. Kawabe, M. Tadokoro, Y. Kojima, Y. Fujisawa, H. Sakurai, Chem. Lett. (1998) 9.
[72] K. Kawabe, M. Tadokoro, A. Ichimura, Y. Kojima, T. Takino, H. Sakurai, J. Am. Chem. Soc. 121 (1999) 7937.
[73] K. Kawabe, T. Suekuni, T. Inada, K. Yamato, M. Tadokoro, Y. Kojima, et al., Chem. Lett. (1998) 1155.
[74] N. Choudhary, D.L. Hughes, U. Kleinkes, L.F. Larkworthy, G.J. Leigh, M. Maiwald, et al., Polyhedron 9 (1997) 1517.
[75] N. Durai, G. Saminathan, J. Clin. Biochem. Nutr. 22 (1997) 31.
[76] C.R. Cornman, T.C. Stauffer, P.D. Boyle, J. Am. Chem. Soc. 119 (1997) 5986.
[77] T.-B. Wen, J.-C. Shi, X. Huang, Z.-N. Chen, Q.-T. Liu, B.-S. Kang, Polyhedron 17 (1998) 331.
[78] W. Tsagkalidis, D. Rodewald, D. Rehder, V. Vergopoulos, Inorg. Chim. Acta 219 (1994) 213.
[79] H. Sakurai, H. Sano, T. Takino, H. Yasui, Chem. Lett. (1999) 913.
[80] L.C.Y. Woo, V.G. Yuen, K.H. Thompson, J.H. McNeill, C. Orvig, J. Inorg. Biochem. 76 (1999) 251.
[81] A. Shaver, J.B. Ng, R.C. Hynes, B.I. Posner, Acta Crystallogr. C 50 (1994) 1044.
[82] A. Shaver, D.A. Hall, J.B. Ng, A-M. Lebuis, R.C. Hynes, B.I. Posner, Inorg. Chim. Acta 229 (1995) 253.
[83] A. Shaver, J.B. Ng, D.A. Hall, B.S. Lum, B.I. Posner, Inorg. Chem. 32 (1993) 3109.
[84] C. Djordjevic, M. Lee, E. Sinn, Inorg. Chem. 28 (1989) 719.
[85] C. Djordjevic, L.G. Gonshor, M.A. Bynum, Inorg. Chim. Acta 79 (1983) 301.
[86] C. Djordjevic, G.L. Wampler, J. Inorg. Biochem. 25 (1985) 51.
[87] A.E. Gerbase, M. Martinelli, L.G. Fagundes, V. Stefani, S.J. Da Silva, J. Coord. Chem. 47 (1999) 451.
[88] D.C. Crans, A.D. Keramidas, H. Hoover-Litty, O.P. Anderson, M.M. Miller, L.M. Lemoine, et al., J. Am. Chem. Soc. 119 (1997) 5447.
[89] P.C. Paul, S.J. Angus-Dunne, R.J. Batchelor, F.W.B. Einstein, A.S. Tracey, Can. J. Chem. 75 (1997) 429.
[90] S.J. Angus-Dunne, P.C. Paul, A.S. Tracey, Can. J. Chem. 75 (1997) 1002.
[91] F. Nxumalo, A.S. Tracey, J. Biol. Inorg. Chem. 3 (1998) 527.
[92] C. Cuncic, S. Desmarais, N. Detich, A.S. Tracey, M.J. Gresser, C. Ramachandran, Biochem. Pharmacol. 58 (1999) 1859.
[93] F.E. Mabbs, D.J. Machin, Magnetism and Transition Metal Complexes, Chapman and Hall, London, 1973.
[94] K.D. Soulti, A. Troganis, A. Papioannou, T.A. Kabanos, A.D. Keramidas, Y.G. Deligiannakis, et al., Inorg. Chem. 37 (1998) 6785.
[95] M. Melchior, S.J. Rettig, K.H. Thompson, V.G. Yuen, J.H. McNeill, C. Orvig, Inorg. Chem., in press.
[96] M. Finnegan, T.G. Lutz, W.O. Nelson, A. Smith, C. Orvig, Inorg. Chem. 26 (1987) 2171.
[97] R.C. Hider, A.D. Hall, Prog. Med. Chem. 28 (1991) 42.

[98] E.T. Clarke, A.E. Martell, J. Reibenspies, Inorg. Chim. Acta 196 (1992) 177.
[99] S.R. Cooper, Y.B. Koh, K.N. Raymond, J. Am. Chem. Soc. 104 (1982) 5092.
[100] M. Chatterjee, M. Maji, S. Ghosh, T.C.W. Mak, J. Chem. Soc. Dalton Trans. (1998) 3641.
[101] P. Ghosh, O.J. D'Cruz, R.K. Narla, F.M. Uckun, Clin. Cancer Res. 6 (2000) 1536.
[102] R.K. Narla, Y.H. Dong, O.J. D'Cruz, C. Navara, F.M. Uckun, Clin. Cancer Res. 6 (2000) 1546.
[103] B.I. Posner, R. Faure, J.W. Burgess, A.P. Bevan, D. Lachance, G. Zhang-Sun, et al., J. Biol. Chem. 269 (1994) 4596.
[104] C. Djordjevic, S.A. Craig, E. Sinn, Inorg. Chem. 24 (1985) 1281.
[105] C. Djordjevic, N. Vuletic, M.L. Renslo, B.C. Puryear, R. Alimard, Mol. Cell. Biochem. 153 (1995) 25.
[106] P. Köpf-Maier, H. Köpf, Chem. Rev. 87 (1987) 1137.
[107] A. Evangelou, S. Karkabounas, G. Kalpouzos, M. Malamas, R. Llasko, D. Stefanou, et al., Cancer Lett. 119 (1997) 221.
[108] R. Llasko, T.A. Kabanos, S. Karkabounas, M. Malamas, A.J. Tasiopoulos, D. Stefanou, et al., Anticancer Res. 18 (1998) 3609.
[109] O.J. D'Cruz, Y. Dong, F.M. Uckun, Biol. Reprod. 60 (1999) 435.
[110] O.J. D'Cruz, A. Vassilev, F.M. Uckun, Biochem. Biophys. Res. Commun. 270 (2000) 21.
[111] M. Pasquali, C. Floriani, A. Chiesi-Villa, C. Guastini, Inorg. Chem. 108 (1986) 7263.
[112] N. Zavellas, N. Klouras, C.P. Raptopoulou, Z. Anorg. Allg. Chem. 622 (1996) 898.
[113] S. Ghose, M.J. May, E.B. Kopp, Annu. Rev. Immunol. 16 (1998) 225.
[114] G.L. Schieven, A.F. Wahl, S. Myrdal, L. Grosmaire, J.A. Ledbetter, J. Biol. Chem. 270 (1995) 20824.
[115] M. Carballo, M. Conde, R. El Bekay, J. Martín-Nieto, M.J. Camacho, J. Monteseirín, et al., J. Biol. Chem. 274 (1999) 17580.
[116] G.L. Schieven, US Patent 5846998, 6 December 1998.
[117] T. Cruz, PCT Int. Appl. WO 9747296, published 18 December 1997.
[118] R. Lehmann, E.D. Schleicher, Clin. Chim. Acta 297 (2000) 135.
[119] I.C. West, Diabetes Med. 17 (2000) 171.
[120] D.C. Ware, W.R. Wilson, W.A. Denny, C.E.F. Rickard, J. Chem. Soc. Chem. Commun. (1991) 1171.
[121] I.G. Fantus, E. Tsiani, Mol. Cell. Biochem. 182 (1998) 109.
[122] C. Hiort, J. Goodisman, J.C. Dabrowiak, Biochemistry 35 (1996) 12354.
[123] E. Tsiani, E. Bogdanovic, A. Sorisky, L. Nagy, I.G. Fantus, Diabetes 47 (1998) 1676.
[124] G.R. Ehring, H.H. Kerschbaum, C.M. Fanger, C. Eder, H. Rauer, M.D. Cahalan, J. Immunol. 164 (2000) 679.