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Vanadium chemistry and biochemistry of relevance for use of vanadium compounds as antidiabetic agents

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Abstract

The stability of 11 vanadium compounds is tested under physiological conditions and in administration fluids. Several compounds including those currently used as insulin-mimetic agents in animal and human studies are stable upon dissolution in distilled water but lack such stability in distilled water at pH 7. Complex lability may result in decomposition at neutral pH and thus may compromise the effectiveness of these compounds as therapeutic agents; Even well characterized vanadium compounds are surprisingly labile. Sufficiently stable complexes such as the VEDTA complex will only slowly reduce, however, none of the vanadium compounds currently used as insulin-mimetic agents show the high stability of the VEDTA complex. Both the bis(maltolato)oxovanadium(IV) and peroxovanadium complexes extend the insulin-mimetic action of vanadate in reducing cellular environments probably by increased lifetimes under physiological conditions and/or by decomposing to other insulin mimetic compounds. For example, treatment with two equivalents of glutathione or other thiols the (dipicolinato)peroxovanadate(V) forms (dipicolinato)oxovanadate(V) and vanadate, which are both insulin-mimetic vanadium(V) compounds and can continue to act. The reactivity of vanadate under physiological conditions effects a multitude of biological responses. Other vanadium complexes may mimic insulin but not induce similar responses if the vanadate formation is blocked or reduced. We conclude that three properties, stability, lability and redox chemistry are critical to prolong the half-life of the insulin-mimetic form of vanadium compounds under physiological conditions and should all be considered in development of vanadium-based oral insulin-mimetic agents. (Mol Cell Biochem **153:** 17–24, 1995)

Key words: vanadium chemistry, vanadium biochemistry, compound stability, compound lability, insulin- mimetic, metabolic involvement

Abbreviations: ADP – adenosine 5′-diphosphate; ATP - adenosine 5′-triphosphate, ADP-V – adenosine 5′-diphosphate-vanadate; bpV – bis(peroxo)oxovanadium(V); (bpV)2 – bis(peroxo)oxovanadium(V) dimer; bpVpic – bis(peroxo)picolinatooxovanadate(V); ^{13}C – carbon-13; EDTA – ethylenediaminetetraacetic acid; EPR – electron paramagnetic resonance; EXSY – exchange spectroscopy; ^{1}H – proton; HSG – glutathione; NAD – β-nicotinamide adenine dinucleotide; NADP – β-nicotinamide adenine dinucleotide phosphate; NADV – β-nicotinamide adenine dinucleotide vanadate; NMR – nuclear magnetic resonance (also referred to as magnetic resonance imaging); pVdipic – (dipicolinato)peroxovanadate(V); Vcit – (citrato)dioxovanadate(V); VEDTA – (ethylenediaminetetraacetato)dioxovanadate(V); Vmalto – bis(maltolato)-oxovanadium(IV); Voxal – bis(oxalato)dioxovanadate(V); ^{51}V – vanadium-51; V_1 – vanadate monomer; V_2 - vanadate dimer; V_4 – vanadate tetramer; V_5 – vanadate pentamer; UV-vis spectroscopy – ultraviolet-visible spectroscopy

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Introduction

In vitro and animal studies show that vanadate and other vanadium compounds increase glucose transport activity and normalize glucose metabolism [1–5]. Furthermore, these insulin-mimetic compounds can be administered orally. Vanadate enhances the phosphoprotein formation which is attributed to either the activation of protein kinases or inhibition of protein phosphatases. Despite the interest in documenting the effects of vanadate on protein kinases, most reports have used indirect methods and studies with purified kinases show weak, if any, interaction of vanadate with kinases as a group of enzymes (reviewed in Refs. [6–8]). Vanadate interacts potently with phosphatases and the inhibition is attributed to a five-coordinate vanadate complex which mimics the transition state of the phosphate ester hydrolysis reaction (reviewed in Refs. [7, 9]). Given the multitude of alternative mechanisms by which vanadate can act at the cellular level consideration of these effects should assist in identification of the undesirable modes of action [6–9]. Since mechanistic information will enable us to optimize insulin-mimetic effects and minimize toxicity, it is appropriate to briefly discuss some alternative modes of action of vanadate in cells.

Vanadate as a phosphate analog is a potent inhibitor for ATPases; the lowest K_i value for the Na^+-K^+-ATPase has been reported to 9 nM thus justifying the considerations of this enzyme system as a major contributor to the biological effects of vanadate [10]. However, the effects of vanadate extend way beyond the ATPases and vanadate as a phosphate analog. Vanadate is believed to enter cells through phosphate transport systems (path a, Fig. 1) [11]. Based on the reactivity of vanadate and the cellular components and metabolites, vanadate is likely to undergo several reactions inside the cell (illustrated in Fig. 1). Since the *in vivo* responses to vanadate represent the cumulative responses to all the vanadium compounds present in the cell, the intracellular form vanadate becomes essential to the overall responses to vanadate. First, vanadate reacts with alcohols to form esters (eq. 1) [12]. Second, vanadate reacts with phosphates to form vanadate-phosphate anhydrides (eq. 2) [13]. In the presence of glutathione (HSG) [14, 15], or ascorbate [16], vanadate is reduced to vanadium(IV). Once administered vanadate eventually reduces to vanadium(IV) depending on intracellular location and cell type [11, 16–20]. Whether the slow formation of vanadium(IV) is related to a slow reaction or a slow penetration of cells by vanadate is not clear [11]. Since free vanadyl cation is stable to only 10^{-7} M at neutral pH, generated vanadium(IV) will combine with available ligands (path f, Fig. 1) [21].

$$H_2VO_4^- + HOR \rightleftarrows ROVO_3H^- + H_2O \quad (1)$$

$$H_2VO_4^- + H_2PO_4^- \rightleftarrows HOVO_2OPO_3H^{2-} + H_2O \quad (2)$$

Because most cellular components contain hydroxyl and/or phosphate groups, vanadate reacts as shown in eq. 1, and 2 with a variety of metabolites. For example, the reaction of vanadate with the 2′-hydroxyl group of the cofactor NAD generates an NADP analog, NADV (path b) [22]. NADV is an excellent cofactor for enzymes such as glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and alcohol dehydrogenase [22]. The presence of NADV could affect the levels of reducing equivalents in the cell, important in maintaining a normal glucose metabolism. This type of mechanism is distinct from the vanadate-induced NADH oxidation by plasma membranes [23]. Organic vanadates have been shown to substitute for organic phosphates in many of the enzymes related to glucose metabolism and could effect these enzymes as well [24, 25]. Other organic vanadates are potent inhibitors of phosphatases [9], ribonucleases [26] and phosphoglucomutase [27, 28]. Formation of a vanadate-protein tyrosine phosphatase complex (path d) is particularly relevant to the vanadate-induced insulin-mimetic effects [9].

Nucleotides are other metabolites that could react with vanadate as shown in eq. 2 and Fig. 2 to form metabolite-conjugates. Specifically, ADP-V is accepted by myosin in place of ATP (path c) [29]. The corresponding GDP-V is accepted by adenylate cyclase in place of GTP and activates this enzyme for synthesis of cAMP (path c) [30]. Analogous processes could involve other metabolites and enzyme systems [7]. Although most of this discussion focuses on vanadate and vanadate derived compounds, vanadium(IV) compounds sometimes cause similar responses (see for example Refs. [22, 25, 31]).

The reactions shown in Fig. 1 underscore the importance of determining the type of intracellular vanadium compounds that form. Vanadate and vanadium compounds have commonly been used in animal studies with little consideration of their chemistry or information on the active species: Do all these compounds convert to a common form? Despite the few lability studies of vanadium complexes, we will here show that three physicochemical properties of vanadium compounds are essential to drug administration; stability, lability and redox properties. In this paper we will describe the aqueous chemistry of relevance to administration of vanadium compounds. Second, studies probing the lability and stability of a series of vanadium compounds are discussed and finally, the redox stability of these complexes are considered.

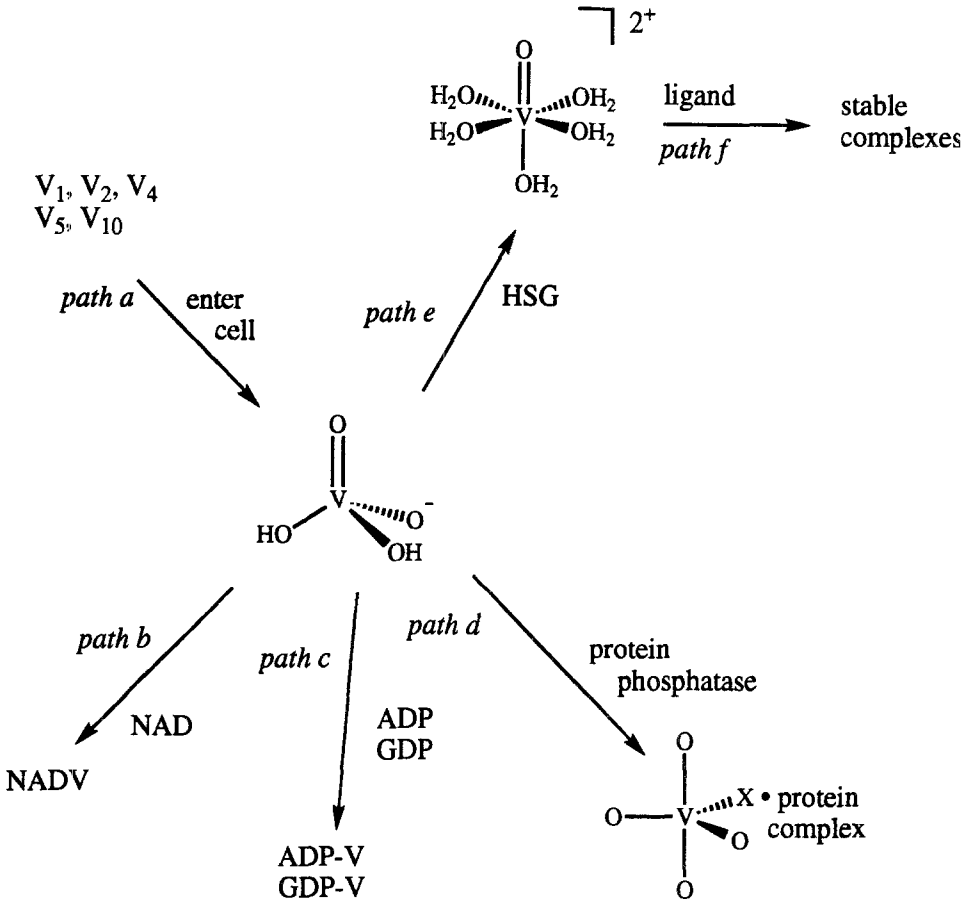

Fig. 1. Intracellular vanadium(V) compounds that can form when vanadate has been administered. Each compound has been found to act as substrate, cofactor or inhibitor for enzymes related to glucose metabolism.

Materials and methods

Materials

The vanadium complexes (see list of abbreviations) were prepared as described previously for bpV [32], $(bpv)_2$ [33], bpVpic [34], Vdipic [35], pVdipic [35], Vmalto [36], VEDTA [37], Vcit [38], and Voxal [39]. The reagents used in these synthesis reactions were purchased from Aldrich and used without further purification unless indicated otherwise.

Stock solutions

Vanadate stock solutions were prepared from sodium orthovanadate (Na_3VO_4) or sodium metavanadate ($NaVO_3$). When pH was adjusted by the addition of HCl, the stock solution immediately turned yellow-orange indicating the presence of vanadate decamer; such solutions were heated or stored until the yellow-orange color disappeared [40]. The pH often changed until the solution was colorless so pH adjustments were repeated until the desired pH was obtained.

Sample preparation of vanadium compounds

All NMR samples were prepared at ambient temperatures and contained 20% D_2O. Solutions were prepared by dissolution of the crystalline compound into double-distilled water, and pH was adjusted with NaOH or HCl. No solution changed color during pH adjustment except for sodium orthovanadate. Some samples were prepared in buffer (HCO_3^-/CO_3^{2-} or Hepes) to maintain constant pH. The Vmalto solution was prepared under nitrogen and the stability studied when the nitrogen was removed. Identical samples for UV-vis spectroscopic studies were prepared in the absence of D_2O. 2-Mercaptoethanol was added to some samples immediately before UV-vis spectroscopy.

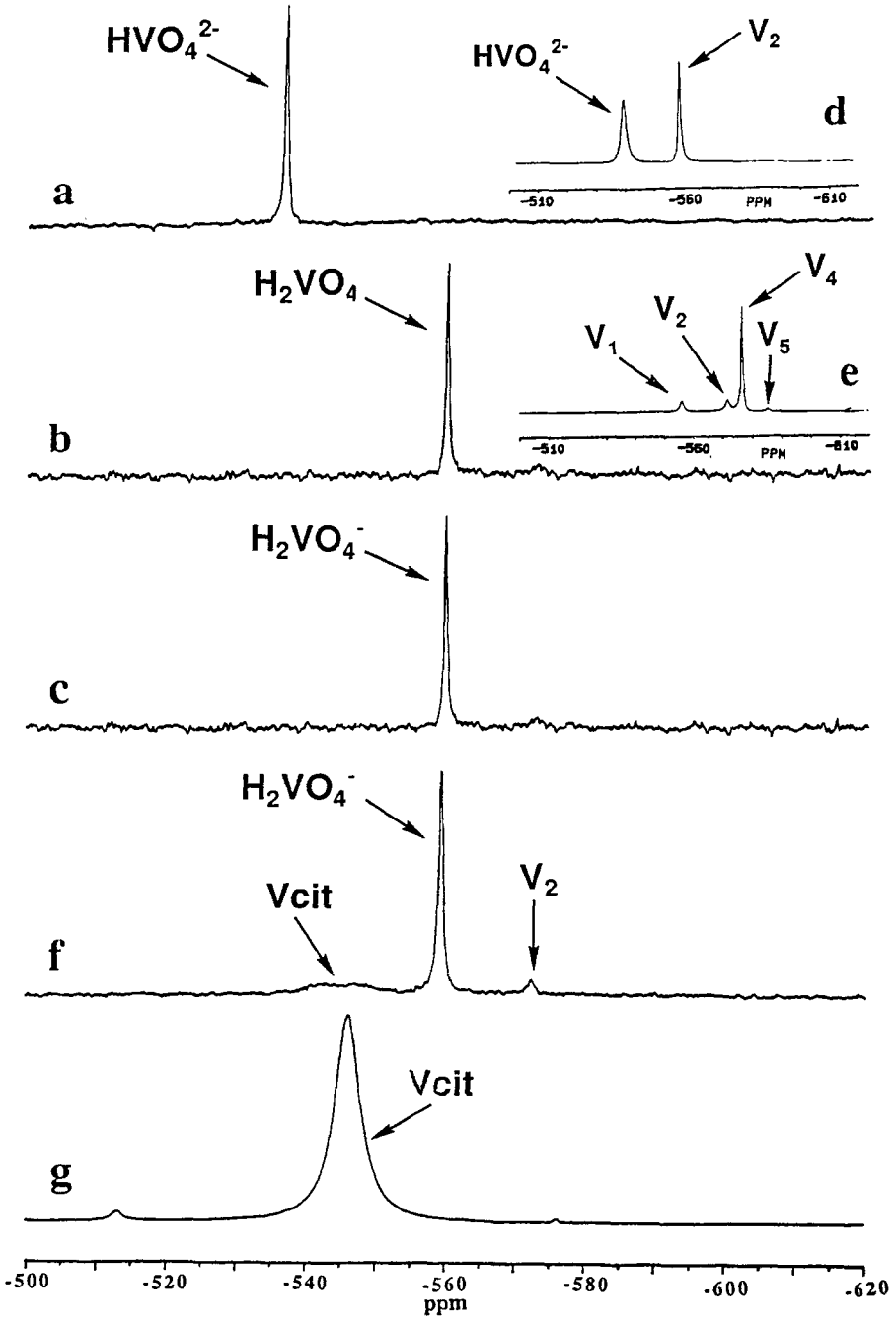

Fig. 2. ^{51}V NMR spectra of vanadate solutions prepared from various sources of vanadate: a) 0.38 mM vanadate prepared from sodium orthovanadate (pH 10.50), b) 0.38 mM vanadate prepared from sodium metavanadate (pH 7.04), c) 0.4 mM colorless vanadate prepared from sodium orthovanadate and adjusted to pH 7.04, d) 10.0 mM vanadate prepared from sodium orthovanadate (pH 12.5), e) 10.0 mM vanadate prepared from sodium metavanadate (pH 7.04), f) 0.38 mM sodium orthovanadate adjusted to pH 7.04 with citric acid, and g) 10.0 mM sodium metavanadate and 40.0 mM citrate at pH 7.04. The data presented were confirmed in duplicate or triplicate experiments.

Spectroscopy

^{51}V NMR, UV-vis and EPR spectroscopy were used to monitor the decomposition of vanadium compounds. Each ^{51}V NMR spectrum took 4.5 min to record using routine parameters [40]. ^{51}V NMR spectroscopy is limited to measuring the vanadium(V) in solution, so to confirm the NMR studies, we used UV-vis spectroscopy (Perkin Elmer kamda 4B) which gives different absorption spectra for the vanadium(V) and vanadium(IV) compounds in solution. Extinction coef-

ficients and/or absorbances for each vanadium derivative were measured first, and then the UV absorbance was used to measure the disappearance and/or appearance at various wavelengths of a specific complex and other components in solution. In some cases EPR spectroscopy was used to measure the vanadium(IV) compounds.

Results and discussion

Vanadate salts

Several vanadate salts are commercially available, but all form the same vanadate anions on dissolution (reviewed in Ref. [40]) (Fig. 2). A solution of sodium orthovanadate in distilled water at 0.38 mM vanadate has a pH of 10.50 and contains mostly HVO_4^- (Fig. 2A). ^{51}V NMR spectroscopy is a convenient method to monitor the form of vanadate in solution since the chemical shift is very sensitive to the form of the vanadium atom (see Ref. [40] and references therein). A solution of 0.38 mM vanadate from sodium metavanadate has pH 7.04 and contains mainly $H_2VO_4^-$ with some HVO_4^{2-} (Fig. 2B). Adjusting the pH of the solution in Fig. 2A will produce solutions with the same anionic form of vanadate as shown in Fig. 2B (Fig. 2C). The spectrum recorded of a stock solution of 10.0 mM sodium orthovanadate (pH 12.5) is shown in Fig. 2D. The additional resonance observed is vanadate dimer (V_2) which hydrolyzes upon dilution or administration [41]. The spectrum recorded of a stock solution of 10.0 mM sodium metavanadate (pH 7.04) contains three additional signals: V_2, V_4 and V_5 which all rapidly hydrolyze to form vanadate upon dilution or administration [41]. The presence of these other forms in stock solutions is therefore of no concern. However, the addition of for example citrate to drinking water for rats (see, for example, Ref. [42]) can be problematic; see ^{51}V NMR spectra in Fig. 2F and 2G of vanadate solutions containing various concentrations of citrate. If possible, additives should be avoided because they often generate new complexes with vanadate and with vanadyl cation.

Stability of vanadium compounds in aqueous solution

The stability of 11 vanadium compounds was measured using ^{51}V NMR, UV and EPR spectroscopy in aqueous solution including compounds previously used as insulin-mimetic agents (vanadate, $VOSO_4$, bpV, bpVpic, pVdipic, Vmalto), and related compounds (VEDTA, Vcit, Voxal, $(bpV)_2$ and Vdipic) (Fig. 3). As shown in Fig. 3A, vanadate, $VOSO_4$, $(bpV)_2$, pVdipic, Vdipic, VEDTA, Vcit and Voxal showed little evidence for decomposition after 4 days when dissolved in distilled water (see Fig. 3A caption for pH information). The results obtained by ^{51}V NMR, EPR and UV-vis spectroscopy agreed in all cases when both carried out. Vmalto decomposed 50% in 1 day, at which level it remained for 4 days. Both bpV and bpVpic completely decomposed within 1 day, however, some (if not all) peroxo compounds are sensitive to light [43], and since peroxovanadium species decompose through radical mechanisms [44] significant variation in decomposition times can be expected depending on purity of bpVpic, water and other solution additives. Some variation was observed even in our hands in repeat decomposition runs particularly in the case of bpVpic; the results shown represent an average of three experiments.

Adjusting the solutions to pH 7 and maintaining this pH by 15 or 20 mM Hepes decreased (or remained the same) the stability of all but one complex (Fig. 3B), bpVpic, which had an increased half-life. The reactions were monitored both in distilled water and in Hepes, and unless indicated, no differences were observed. At pH 7 Voxal and $VOSO_4$ hydrolyzed completely within 5 min, and 30–50% of the vanadyl cation oxidized to vanadate within 4 days. Three compounds bpV, pVdipic and Vmalto hydrolyzed completely within 2 days. The decomposition rate of bpVpic in Hepes at pH 7 decreased, and only after approximately 2 days, the compound began to decompose rapidly as observed for radical decomposition mechanisms [44]. Within 5 min 70% of Vdipic decomposed and 15% of Vcit decomposed; both remained at these levels after 4 days. In contrast, vanadate, $(bpV)_2$, and VEDTA complexes showed no change for 4 days at neutral pH.

Most of the vanadium complexes examined are stable in solution for days when dissolved in distilled water. However, if these compounds are administered orally as insulin-mimetic agents, the stability of the compounds at acidic pH (not shown here) and neutral pH is of concern. We have shown that the stability of most compounds examined here decrease at neutral pH, suggesting that intracellular life-times of these particular compounds are limited.

Lability of vanadium compounds in aqueous solution

Little information is available on the lability of vanadium complexes including insulin-mimics under physiological conditions. Given space limitations, we will not provide experimental evidence here for lability of even well-known vanadium compounds. Suffice to say, that despite the documentation by ^{51}V NMR spectroscopy of the stability of a particular vanadium compound, such complexes can be exceedingly labile [45, 46]. This point is important, because labile complexes will convert to vanadate under physiological conditions which accesses the multitude of reactions shown in Fig. 1 for vanadate. Lability is an important property for vanadium compounds used in diabetic drug therapy and should be considered.

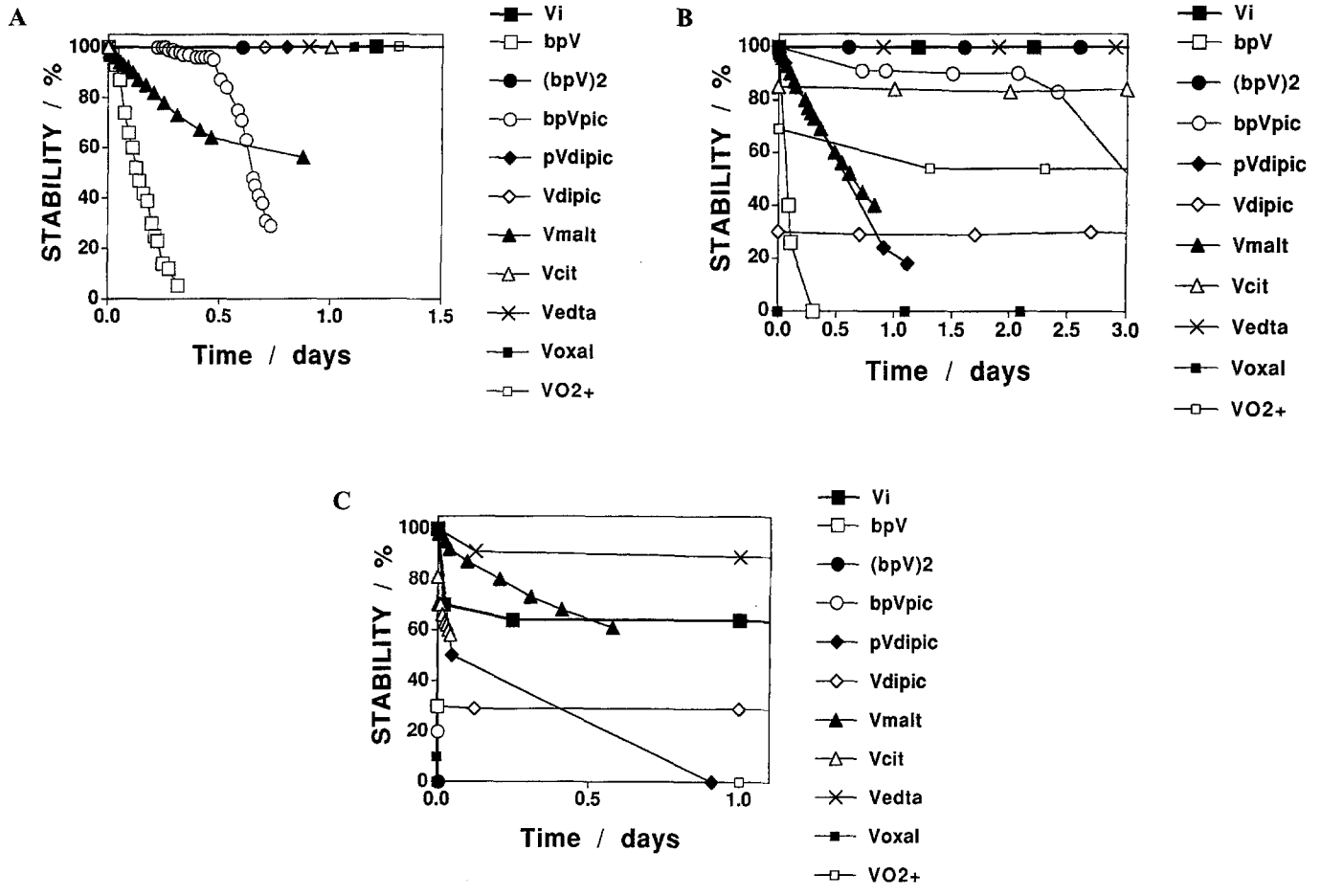

Fig. 3. The stability of a series of vanadium compounds dissolved in: A) distilled water, B) distilled water containing 15 or 20 mM Hepes at 7.0 (± 0.2). Hepes was added to avoid changes in pH during decomposition. C) distilled water containing 15–20 mM Hepes added 2 equivalents of 2-mercaptoethanol at pH 7.0 (± 0.2). The measurement was carried out using both ^{51}V NMR spectroscopy (2 and 10 mM solutions), UV spectroscopy (10 mM solutions) and in some/other cases confirmed by EPR spectroscopy. Specifically in the thiol studies, the thiol absorbence spectra often overlap with the original vanadium compound necessitating us to monitor the formation of new presumably vanadium(IV) species at higher wavelengths. The wavelength and the absorbence for the species examined by UV spectroscopy are as follows: $NaVO_3$ (203 nm, 0.48 mM, at 100% A = 2.09), $VOSO_4$ (204 nm, 0.48 mM, at 100% A = 0.331), Vcit (569 nm, 10 mM, at 100% A = 0.0, at 0% A = 0.187), Vmalto (877 nm, 4 mM, at 100% A = 0.116), Voxal (800 nm, 10 mM, at 100% A = 0.0, at 0% A = 0.180), Vdipic (860 nm, 10 mM, at 100% A = 0.0, at 0% A = 0.349), VEDTA (779 nm, 10 mM, at 100% A = 0.0, at 0% A = 0.244). The symbols for each compound and the pH of the solution represented in Fig. 3A are indicated: vanadate, 7.1 (■), bpV, 6.8 (□), $(bpv)_2$, 6.6 (●), bpVpic, 7.3 (○), pVdipic, 6.2 (◆), Vdipic, 5.4 (◊), V-malto, 5.3 (▲), V cit, 3.3 (Δ), VEDTA, 5.9 (x), V-oxal, 5.1 (■) and vanadyl cation (VO^{2+}), 3.5 (□).

Stability of vanadium compounds in reducing intracellular environments

In Fig. 3C we follow the decomposition of 11 vanadium complexes in the presence of 2 equivalents of 2-mercaptoethanol (see below for glutathione). Vanadate reduces in the presence of a large excess of thiols and these findings were confirmed in this study [14, 15]. However, 2 mM vanadate in the presence of 15 mM Hepes and 4 mM 2-mercaptoethanol formed 23% of a new vanadium(V) complex (–356 ppm) whereas 65% remained as vanadate. After 4 days only 50% of the vanadate remained. VOSO4 immediately formed EPR silent complexes of which 30–40% were vanadium(V) compounds and included the new complex at -356 ppm. This solution remained stable for 4 days.

Four other compounds were immediately reduced by the thiol: bpV, $(bpV)_2$, bpVpic, and Voxal. Vcit completely decomposed within 12 h and the Vmalto decomposed to 50% after 1 day. Upon dissolution at pH 7 only 30% of Vdipic remained, and slow reduction decreased this concentration to 25% after 4 days. The pVdipic converted to Vdipic and vanadate upon treatment with 2 equivalents of 2-mercaptoethanol within 12 h, consistent with reaction of the hydrogen peroxide with the thiol. VEDTA, alone, persisted for 4 days at 90%.

As anticipated, most vanadium(V) compounds were not stable in the presence of glutathione and other thiols. The reduction was, in some cases, very sensitive to the ratio of

thiol to vanadium compound; low ratios would prolong the lifetime of the vanadium compound. Studies were carried out with glutathione, but with this thiol the reduction occurred faster than with 2-mercaptocthanol. However, the fact VEDTA is stable in the presence of 2-mercaptoethanol was very encouraging and probably in part due to the very low equilibrium concentration of vanadate as well as the complex's redox potential. The pVdipic, on the other hand, formed Vdipic when reacting with thiol. Although Vdipic may not have the same insulin-mimetic activities as pVdipic [5], this reactivity pattern prolongs the presence of insulin-mimetic agents under physiological conditions. Further examination of vanadium compounds with desirable insulin mimetic properties could lead to identification of vanadium compounds with enhanced insulin-mimetic responses.

Conclusion

Three key properties, stability, lability and redox chemistry, are critical to the insulin mimetic action of vanadium compounds. These insulin-mimetic effects presumably result from interactions of vanadium(V) (and perhaps vanadium(IV)) compounds with metabolites and other cellular components. Observed stability of a vanadium compound in stock solutions does not imply stability after administration and uptake by cells. The stability under the latter conditions should specifically be examined. The reactivity of vanadate and the other vanadium compounds will affect the compounds insulin-mimetic properties. Exploring compounds with increased stability and modified reactivity pattern could play an important role in development of insulin mimetic agents. For example, despite the lability of the VEDTA complex, its rate of reduction by thiols is slow, which should prevent many of the intracellular reactions observed with vanadate. Alternatively, thiols react with compounds such as bpVdipic to form Vdipic and vanadate thus generating vanadium compounds with continued insulin mimetic action. By both these strategies compounds can extend their life-times and effects under physiological conditions. Recognizing the complex aqueous vanadium chemistry and information on stable compounds and compounds with altered reactivity pattern will facilitate the development of vanadium compounds for therapeutic use as oral insulin substitutes.

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