Dalton Transactions

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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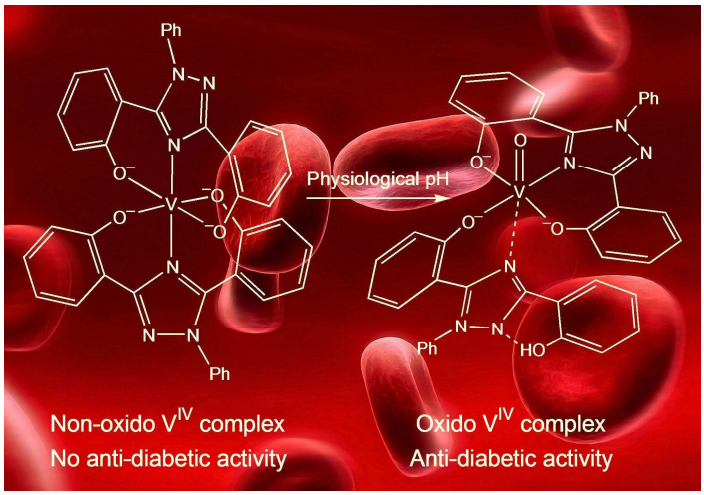
BRIEF. The transformation of non-oxido V^{IV} to oxido V^{IV} species at the physiological conditions is necessary to exhibit the pharmacological anti-diabetic activity.

SYNOPSIS GRAPHIC



Dalton Transactions RSCPublishing

ARTICLE

Structural and redox requirements for the action of anti-diabetic vanadium compounds

Cite this: DOI: 10.1039/x0xx00000x

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Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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This study presents the first systematic investigation of the anti-diabetic properties of non-oxido V^{IV} complexes. In particular, the insulin-mimetic activity of $[V^{IV}(taci)_2]^{4+}$, $[V^{IV}(inoH_{-3})_2]^{2-}$, $[V^{IV}(dhab)_2]$, $[V^{IV}(hyph^{Ph})_2]$, $[V^{IV}(cat)_3]^{2-}$ and $[V^{IV}(pdbh)_2]$ – where taci is 1,3,5-triamino-1,3,5-trideoxy-*cis*-inositol, ino is *cis*-inositol, H_2dhab is 2,2′-dihydroxyazobenzene, H_2hyph^{Ph} is 3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazole, H_2cat is catechol and H_2pdbh is pentan-2,4-dione benzoylhydrazone – was evaluated in terms of free fatty acids (FFA) release. Among the six compounds examined, only $[V^{IV}(pdbh)_2]$, $[V^{IV}(cat)_3]^{2-}$ and $[V^{IV}(hyph^{Ph})_2]$, which at the physiological pH convert to the corresponding $V^{IV}O$ complexes, were found to exhibit a significant insulin-mimetic activity compared to $VOSO_4$. In contrast, $[V(taci)_2]^{4+}$, $[V(inoH_{-3})_2]^{2-}$ and $[V(dhab)_2]$, which at pH 7.4 keep their 'bare' non-oxido structure, did not cause any inhibition of FFA. The results, therefore, suggest that a $V^{IV}O$ functionality is necessary for vanadium complexes to exhibit anti-diabetic effects. This agrees with the notion that the biotransformations of V compounds in the organism are more important than the nature of the species.

1. Introduction

Over the last fifteen years a number of anti-diabetic vanadium compounds have been reported.[1] Most of these complexes contain an oxidovanadium(IV) species, which appears to be the most stable form in reducing biological environments. A $V^{IV}O$ species is presumably the form transported in the blood plasma regardless of the initial oxidation state of the complex administered.[2] *In vivo* EPR studies monitoring blood circulation in rats showed that when vanadium is administered as $VOSO_4$, at least 90% is present in oxidation state +IV in nearly all organs and thus V^{IV} is the focus of this work.[3] The presence of reducing agents such as ascorbate, catecholamines and cysteine and the binding of $V^{IV}O^{2+}$ ion to the bioligands of plasma stabilizes the +IV state and prevents its oxidation.[4] Furthermore, $V^{IV}O$ salts (e.g. $VOSO_4$) and complexes are effective, well tolerated, and produce a significant glucose-lowering in all the animal models of diabetes.[5] In particular, phase I and II clinical trials of $VOSO_4$[6] and coordination compound $[VO(ethylmaltolato)_2]$ (BEOV) have been completed.[7] Because at the physiological conditions, coordination complexes hydrolyse and undergo biotransformation in the blood plasma,[2b, 8, 9, 10] the active insulin-enhancing species remains elusive despite the countless studies exploring this controversial question. The issue arises because of the difficulty in identifying the active V species, which is related to the low concentration needed to exhibit the pharmacological action, the rich chemistry and potential short lifetime of active species under physiological conditions.

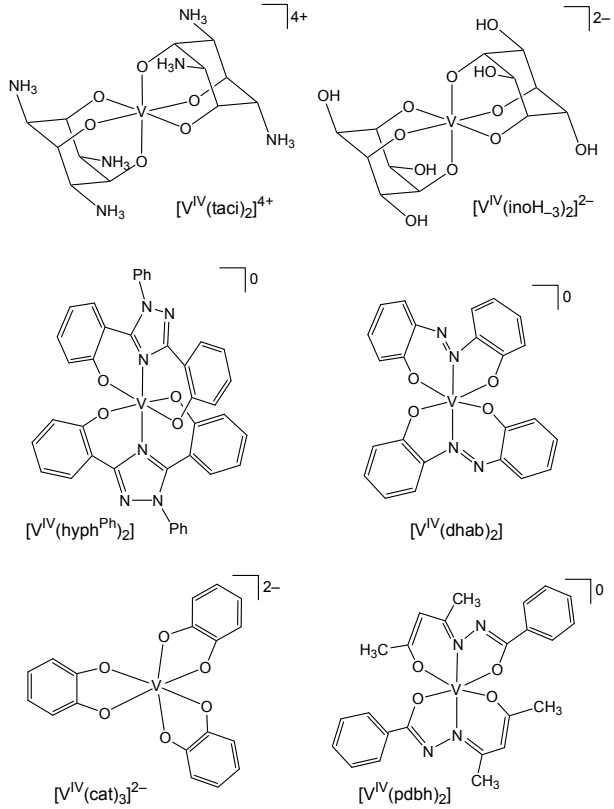
The anti-diabetic effects of the wide range of V compounds investigated are generally attributed to the inhibition of protein tyrosine phosphatases (PTPs). Phosphatase inhibition prevents the dephosphorylation of insulin receptor substrates (IRS), keeping the signal transduction pathways and the transport of glucose into the cell intact.[5, 11] An alternative mode of action involves the interaction with the cell and/or the cell membrane and affects membrane mobility and rigidity.[12] Since the form present under the physiological conditions depends on the interaction with low molecular mass bioligands and proteins of the plasma and, in general, on the hydrolytic and redox biotransformation in the blood and cell, the active V species in the organism is not readily identified.[1e, 11a, 12-13] However, data supporting the possibility that these compounds are important to the redox state of the cells have been discussed by several groups.[13-14] Since almost all studies have been done with V≡O[15] compounds, the importance of the transformation from a 'bare' V to a V≡O species has rarely been studied.

Non-oxido or 'bare' V^{IV} complexes are hexa-coordinated species without the V≡O bond. The number of such 'bare' compounds remains relatively scarce compared to that of $V^{IV}O$ complexes due to the stability of V≡O bond in $V^{IV}O^{2+}$ ion. These species are a unique group of vanadium compounds,

because they have the potential to undergo chemistry not generally possible for other $V^{IV}O$, $V^{V}O$ or $V^{V}O_2$-containing complexes.[5, 11]

Although also vanadium(III) compounds are generally 'bare' complexes, this oxidation state has not been commonly explored as anti-diabetic compounds. As a result, only a few reports on insulin-enhancing V^{III} have been reported.[16, 17] These systems belong to the class of maltolate and dipicolinate compounds and readily form $V^{IV}O$ complexes upon oxidation.

This study presents the first systematic investigation of the anti-diabetic properties of non-oxido V^{IV} complexes shown in Scheme 1. In particular, V^{IV} compounds with 1,3,5-triamino-1,3,5-trideoxy-*cis*-inositol (taci), *cis*-inositol (ino), 2,2'-dihydroxyazobenzene (H_2dhab), 3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazole (H_2hyph^{Ph}), catechol (H_2cat) and pentan-2,4-dione benzoylhydrazone (H_2pdbh) were prepared and their insulin-mimetic activity was evaluated by *in vitro* experiments. The following studies led to a novel hypothesis regarding the mode of action of the anti-diabetic V compounds.



Scheme 1 Structure of non-oxido V^{IV} complexes studied in this work. The notation $inoH_{-3}$ indicates that the ligand has lost three protons with respect to the fully protonated form in water.

2. Experimental and Computational Section

2.1. Preparation of the non-oxido V^{IV} compounds

$[V(dhab)_2]$,[18] $[V(hyph^{Ph})_2]$,[19] $[V(taci)_2](SO_4)_2\cdot 4H_2O$,[20] $Na_6[V(inoH_{-3})_2](SO_4)_2\cdot 2H_2O$,[21] $[V(pdbh)_2]$ [22] and $(Et_3NH)_2[V(cat)_3]\cdot CH_3CN$[23] were synthesized following the procedure reported in the literature. The notation $inoH_{-3}$ indicates that the ligand has lost three protons with respect to the fully protonated form in water. The purity of the V^{IV} compounds was determined through elemental analysis.

$[V(dhab)_2]$. Anal. Calcd for $C_{24}H_{16}N_4O_4V$ (475.39): C, 60.13; H, 4.20; N, 11.69%. Found: C, 60.21; H, 4.11; N, 11.70%.

$[V(hyph^{Ph})_2]$. Anal. Calcd for $C_{42}H_{34}N_6O_6V$ (705.62): C, 68.09; H, 3.71; N, 11.91%. Found: C, 68.18; H, 3.79; N, 11.80%.

$[V(taci)_2](SO_4)_2\cdot 4H_2O$. Anal. Calcd for $C_{12}H_{38}N_6O_{18}S_2V$ (669.54): C, 21.53; H, 5.72; N, 12.55%. Found: C, 21.49; H, 5.82; N, 12.49%.

$Na_6[V(inoH_{-3})_2](SO_4)_2\cdot 2H_2O$. Anal. Calcd for $C_{12}H_{22}O_{22}Na_6S_2V$ (771.30): C, 18.69; H, 2.87%. Found: C, 18.81; H, 2.95%.

$[V(pdbh)_2]$. Anal. Calcd for $C_{24}H_{24}O_4N_4V$ (483.42): C, 59.63; H, 5.00; N, 11.59%. Found: C, 59.56; H, 4.89; N, 11.47%.

$(Et_3NH)_2[V(cat)_3]\cdot CH_3CN$. Anal. Calcd for $C_{32}H_{47}O_6N_3V$ (620.68): C, 61.92; H, 7.63; N, 6.77%. Found: C, 61.77; H, 7.52; N, 6.68%.

2.2. Spectroscopic and analytical measurements

Anisotropic EPR spectra were recorded with an X-band (9.15 GHz) Varian E-9 spectrometer on frozen solutions at 100 K. The values of A were calculated through simulations with the computer program Bruker WinEPR SimFonia.[24] The estimated error in A is $\pm 0.3\times10^{-4}$ cm^{-1}. The frequency was calibrated with diphenylpicrylhydrazyl radical (dpph), whose g value is 2.0036 (see Fig. 2).

For all the complexes, IR spectra were recorded in the range 4000-600 cm^{-1} with a Jasco FT/IR-480Plus spectrometer using KBr disks. The absence of the stretching frequency $\nu_{V\equiv O}$ in the range 900-1000 cm^{-1} indicates the formation of 'bare' V^{IV} species without the triple bond V≡O. Elemental analysis (C, H, N) was carried out with a Perkin-Elmer 240 B elemental analyzer.

2.3. Electrochemistry

Cyclic voltammograms on the systems formed by V^{IV} with H_4anth, H_2hyph^{Ph} and H_2pdbh were recorded with an electrochemical analyzer CH Instruments 600 B at room temperature (25 ± 1 °C) in H_2O (systems with H_4anth and H_2pdbh) or in a mixture H_2O/MeOH 60:40 v/v (system containing H_2hyph^{Ph}) with 0.5 M KNO_3 at the same experimental conditions (pH and ligand to metal molar ratio) used to record EPR spectra shown in Fig. 2. The total V^{IV} concentration was 2 mM. An Au working electrode, a Pt wire counter electrode and an Ag/AgCl reference were employed. All potentials were calculated relative to the Ag/AgCl electrode (0.20 V *vs.* normal hydrogen electrode, NHE), with a value of +0.26 V for the $[Fe(CN)_6]^{3-/4-}$ couple (0.01 M NaOH). Voltammograms were obtained at scan rates between 0.01 and 1 V s^{-1}. Redox potentials were determined with a precision of ±

0.01 V. The experimental conditions for the electrochemistry measurements of the reported systems (see Table 3) were detailed in the Refs. [20-21], [23], [25]; however, comparisons are subject to the variations in the experimental conditions.[26]

2.4. Toxicity

The toxicity of the vanadium compounds was evaluated on rat adipocytes using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, based on the reduction in the living cells of MTT to formazan, which is determined spectrophotometrically at 570 nm.[27]

2.5. Insulin-mimetic activity

The insulin-mimetic activity of the non-oxido V^{IV} complexes was estimated by *in vitro* experiments on the inhibition of release of free fatty acid (FFA) from isolated rat adipocytes treated with epinephrine.[28] Male Wistar rats were sacrificed under anesthesia with diethyl ether. The adipose tissues were removed, chopped with scissors and digested with collagenase for 60 min at 37 °C in Krebs Ringer bicarbonate buffer (120 mM NaCl, 1.27 mM $CaCl_2$, 1.2 mM $MgSO_4$, 4.75 mM KCl, 1.2 mM KH_2PO_4, 24 mM $NaHCO_3$; pH 7.4), containing 2% bovine serum albumin. The obtained adipocytes were then separated from undigested tissues by filtration through nylon mesh and washed three times. The vanadium complexes were dissolved in 6 µL of DMSO at various concentrations (final concentration 0.1, 0.5 and 1 mM) and 24 µL of saline. 30 µL of this solution, 10 µL of glucose (final concentration 5 mM) and 5 µL of ascorbic acid (final concentration 1 mM), which is added to prevent auto-oxidation of the complexes during the incubation, were added to 240 µL of the isolated adipocytes, and the resulting suspension were incubated at 37 °C for 30 min. Finally, 15 µL of epinephrine solution (final concentration 10 µM) were added to the reaction mixture and the resulting solution was incubated at 37 ºC for 180 min. The reaction was stopped when soaked in ice water, and the mixture was centrifuged at 3,000 rpm for 10 min. FFA levels in the outer solution of the cell were determined using an FFA kit (NEFA C-test WAKO, Wako Pure Chemicals). *In vitro* insulin-mimetic activity of the complexes was evaluated by the IC_{50} value, which defines the concentration of the metal complex required to inhibit 50% of the FFA release from the isolated rat adipocytes treated with epinephrine. Each experiment was repeated three times and average results are presented.

All animal experiments in the present study were approved by the Experimental Animal Research of Kyoto Pharmaceutical University (KPU) and were performed according to the Guideline for Animal Experimentation of KPU.

2.6. DFT calculations

The geometries of V^{IV} complexes were optimized in the gas phase with Gaussian 09 (revision C.01) software,[29] using the hybrid exchange-correlation functional B3P86 and the basis set 6-311g. This choice ensures a good degree of accuracy in the prediction of the structures of first-row transition metal complexes,[30] and in particular of vanadium compounds.[31]

The ^{51}V $\boldsymbol{A}$ tensor was calculated using the functional BHandHLYP and 6-311g(d,p) basis set with Gaussian 09, or using the functional PBE0 and VTZ basis set with ORCA software,[32] according to the procedures published in the literature.[33]

3. Results and discussion

The six V^{IV} complexes studied have different coordination modes (VO_6 and VO_4N_2), electric charge (positive, neutral and negative), geometry (octahedral and trigonal prismatic), and – in the case of tridentate ligands – isomerism (*meridional* or *facial*). The compounds have been characterized through elemental analysis, EPR and IR spectroscopy; in particular, the values of ^{51}V hyperfine coupling constants along the *x* or *z* axis (A_x or A_z) are lower than 145×10^{-4} cm^{-1},[34] whereas in the IR spectra the stretching frequency of V≡O bond ($\nu_{V\equiv O}$, which usually falls in the range 900-1000 cm^{-1}) is not detected. These features indicate that the complexes do not contain the $V^{IV}O^{2+}$ ion but the 'bare' V^{IV} form.

Insulin promotes the synthesis of lipids in the lipogenesis but inhibits the release of free fatty acids (FFA) in lipolysis. The insulin-mimetic action of a V compound can, therefore, be evaluated as the degree of enhancement of glucose incorporation into the cells as well as the inhibition of the release of free fatty acid from the cells.[1c, 35]

First of all, the V compounds investigated did not show any toxicity when incubated at concentrations of 0.1 mM. Cytotoxicity was observed when treatments were done at concentration above 1 mM for some of the compounds and this is in line with what was reported in the literature for other vanadium complexes.[36] Fig. 1 shows the effects of the V^{IV} compounds on the FFA release. The free ligands did not show any insulin-mimetic activity. The effects of three of the six complexes – $[V(hyph^{Ph})_2]$, $[V(pdbh)_2]$ and $[V(cat)_3]^{2-}$ follow dose-response curves in the range 0.1-1.0 mM. The IC_{50} values were calculated as averages from three measurements from the data in Fig. 1 and are reported in Table 1.

Based on IC_{50} values, $[V(pdbh)_2]$ and $[V(cat)_3]^{2-}$ exhibit a significant insulin-mimetic activity compared to $VOSO_4$, whereas the activity of $[V(hyph^{Ph})_2]$ is slightly lower than $VOSO_4$ (Table 1). Instead, three non-oxido V^{IV} compounds, – $[V(taci)_2]^{4+}$, $[V(inoH_{-3})_2]^{2-}$ and $[V(dhab)_2]$ – did not inhibit of FFA release.

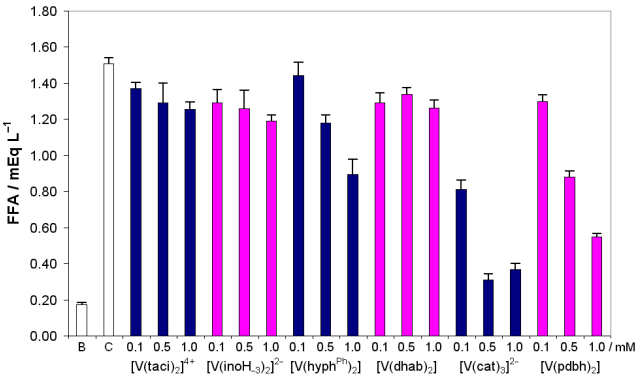


Fig. 1 Inhibitory effects of non-oxido V^{IV} complexes on FFA release from rat adipocytes treated with epinephrine. B and C indicate blank (cells only) and control (cells plus 10 μM of epinephrine), respectively. Data are expressed as the means ± SDs for the three experiments.

Table 1 IC_{50} values for the inhibition of FFA release of non-oxido V^{IV} complexes and the free ligands.[a]

Species	IC_{50} (complex) / mM	IC_{50} (ligand) / mM
$VOSO_4$	0.74	*b*
$[V(taci)_2]^{4+}$	*b*	*b*
$[V(inoH_{-3})_2]^{2-}$	*b*	*b*
$[V(dhab)_2]$	*b*	*b*
$[V(hyph^{Ph})_2]$	2.40	*b*
$[V(cat)_3]^{2-}$	0.11	*b*
$[V(pdbh)_2]$	0.60	*b*

[a] Results are averages of three measurements. [b] No inhibition of FFA release was observed up to 1 mM.

Anisotropic EPR spectra recorded at physiological pH on the systems formed by six ligands studied are shown in Fig. 2 and allow the characterization of the solution structure of V compounds. In the systems with H_2pdbh and H_2hyph^{Ph} derivatives (such as H_3hyph^{S}, see Scheme 2) $V^{IV}O$ complexes with stoichiometry VOL_2H (L = pdbh, hyph^{S}) are formed,[19] whereas in the system with H_2cat $[VO(cat)_2]^{2-}$ exists.[37] In particular, in the $V^{IV}O$ species of of H_2pdbh and H_3hyph^{S} one ligand is tridentate with the (O^-, N, O^-) donor set and the second is bidentate with the (O^-, N) set and one -OH group still protonated.[19]

The transformation of a V^{IV} into a $V^{IV}O$ species can be followed by EPR spectroscopy. In particular, a significant increase of the highest value of the ^{51}V anisotropic hyperfine coupling constant, from 95-145 to 155-165×10^{-4} cm^{-1} is observed, in agreement with what is reported in the literature;[38] the results are confirmed by DFT calculations of the ^{51}V *A* values (Table 2). We conclude that three of the six V^{IV} complexes convert to $V^{IV}O$ species. The transformation of $[V(hyph^{Ph})_2]$, $[V(pdbh)_2]$ and $[V(cat)_3]^{2-}$ into the corresponding $V^{IV}O$ species – $[VO(hyph^{Ph})_2H]^-$, $[VO(pdbh)_2H]^-$ and $[VO(cat)_2]^{2-}$, respectively – at pH 7.4 are shown in Scheme 3. In the systems containing a H_2dhab derivative (H_4anth, Scheme 2), ino and taci, instead, only the non-oxido V^{IV} species exist in aqueous solution (Fig. 2).[18, 20-21]

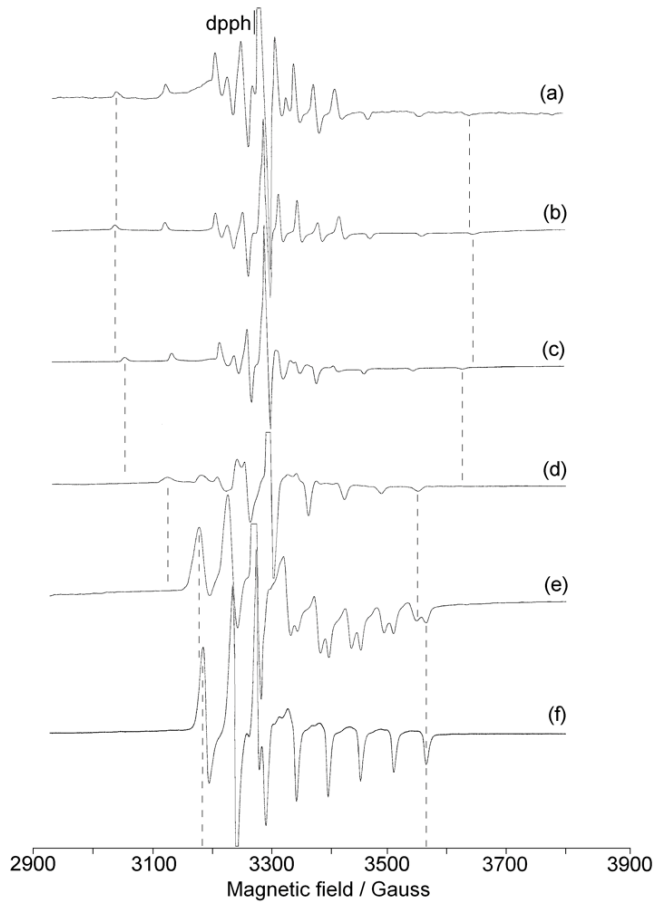
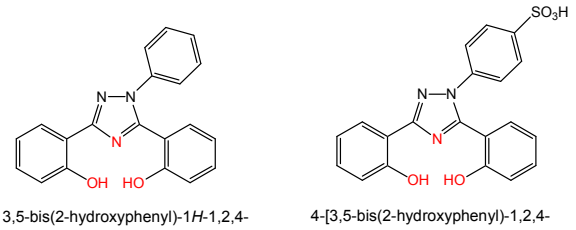


Fig. 2 Anisotropic EPR spectra recorded in an aqueous solution at pH 7.4 and 100 K in the systems formed by V^{IV} with: (a) H_2pdbh, (b) H_3hyph^{S}, (c) H_2cat, (d) H_4anth, (e) ino and (f) taci. H_3hyph^{S} and H_4anth are two derivatives (more soluble in water) of H_2hyph^{Ph} and H_2dhab, see Scheme 2. With the dotted lines the M_I = -7/2 and 7/2 resonances of $V^{IV}O$ and V^{IV} species are shown. It can be seen that, for the non-oxido V^{IV} species (traces d-f), the value of ^{51}V A_i (A_i = A_x or A_z) is significantly lower than that of $V^{IV}O$ complexes (traces a-c). Diphenylpicrylhydrazyl (dpph) is used as a standard field marker (g_{dpph} = 2.0036).



3,5-bis(2-hydroxyphenyl)-1*H*-1,2,4-triazole (H_2hyph^{Ph})

4-[3,5-bis(2-hydroxyphenyl)-1,2,4-triazol-1-yl]-sulphonic acid (H_3hyph^{S})

2,2'-dihydroxyazobenzene (H_2dhab)

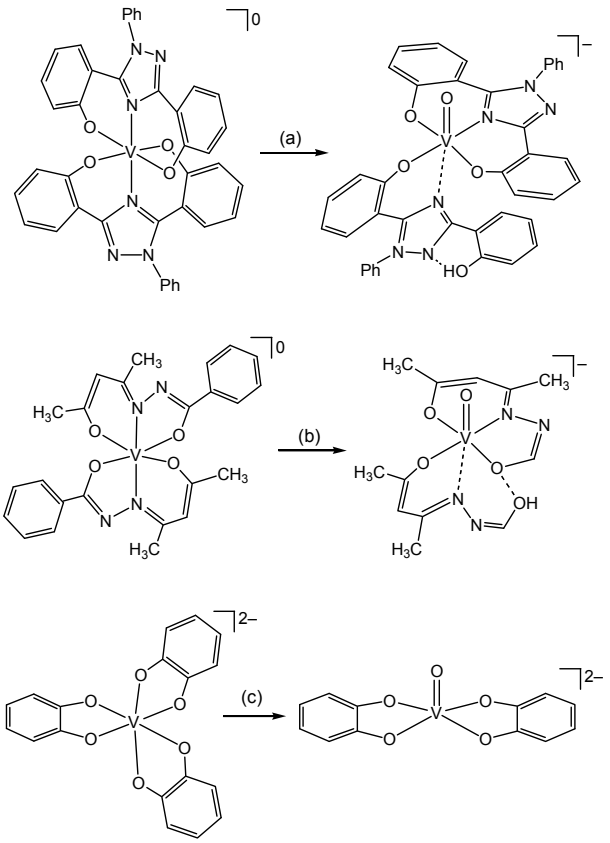
Anthracene chrome red A (H_4anth)

Scheme 2 Structure of H_2hyph^{Ph} and H_2dhab and their derivatives H_3hyph^{S} and H_4anth, see refs. [18] and [19]. H_3hyph^{S} and H_4anth are more soluble in water than H_2hyph^{Ph} and H_2dhab. The coordinating groups are shown in red.

Table 2 EPR parameters of non-oxido V^{IV} complexes.[a]

Species	g_x	g_y	g_z	A_x	A_y	A_z	$A_i^{calcd\ b}$	$A_i^{calcd\ c}$
$[V(taci)_2]^{4+}$	1.902	1.907	1.988	-99.4	-97.2	16.0	-78.1	-89.6
$[V(inoH_{-3})_2]^{2-}$	1.906	1.909	1.986	-99.1	-96.2	24.8	-75.6	-87.5
$[V(dhab)_2]$	1.951	1.962	1.982	-122.4	-52.8	-18.0	-108.8	-114.6
$[V(hyph^{Ph})_2]$ [d]	1.967	1.974	1.943	-55.8	-52.4	-143.2	-127.5	-133.3
$[V(cat)_3]^{2-}$ [e]	1.937	1.937	1.991	-107.0	-107.0	-14.0	-89.2	-104.3
$[V(pdbh)_2]$ [f]	1.950	1.960	1.984	-124.8	-48.6	-17.2	-110.1	-120.4

[a] Values of A reported in 10^{-4} cm^{-1}. [b] Values obtained with Gaussian (A_i is A_x or A_z). [c] Values obtained with ORCA (A_i is A_x or A_z). [d] The value of A_z for the $V^{IV}O$ complex $[VO(hyph^{Ph})_2H]^{3-}$ at pH 7.4 is -162.5×10^{-4} cm^{-1} (A_z^{calcd} -158.1×10^{-4} cm^{-1} with Gaussian and -156.1×10^{-4} cm^{-1} with ORCA). [e] The value of A_z for the $V^{IV}O$ complex $[VO(cat)_2]^{2-}$ at pH 7.4 is -155.0×10^{-4} cm^{-1} (A_z^{calcd} -148.5×10^{-4} cm^{-1} with Gaussian and -145.9×10^{-4} cm^{-1} with ORCA). [f] The value of A_z for the $V^{IV}O$ complex $[VO(pdbh)_2H]^{3-}$ at pH 7.4 is -161.6×10^{-4} cm^{-1} (A_z^{calcd} -157.6×10^{-4} cm^{-1} with Gaussian and -156.3×10^{-4} cm^{-1} with ORCA).



Scheme 3 Transformation of $[V^{IV}(hyph^{Ph})_2]$ (a), $[V^{IV}(pdbh)_2]$ (b) and $[V^{IV}(cat)_3]^{2-}$ (c) at pH 7.4 into the corresponding $V^{IV}O$ species. The complete reactions are $[V^{IV}(hyph^{Ph})_2] + H_2O \rightarrow [V^{IV}O(hyph^{Ph})_2H]^- + H^+$ (a), $[V^{IV}(pdbh)_2] + H_2O \rightarrow [V^{IV}O(pdbh)_2H]^- + H^+$ (b) and $[V^{IV}(cat)_3]^{2-} + H_2O \rightarrow [V^{IV}O(cat)_2]^{2-} + H_2cat$ (c).

The non-oxido V^{IV} complexes with insulin-mimetic activity are $[V(hyph^{Ph})_2]$, $[V(cat)_3]^{2-}$ and $[V(pdbh)_2]$. These compounds according to pH-potentiometric and spectroscopic data underwent hydrolytic reactions around the physiological pH and the V^{IV} convert to the $V^{IV}O$ species. A careful examination suggests that when $V^{IV}O$ species form from the non-oxido complexes, FFA release is revealed. However, no FFA release is observed when only the 'bare' V^{IV} species are present in a solution at pH 7.4. Considering the thermodynamic stability of V compounds and their transformations under physiological conditions the following trend emerges: the anti-diabetic effects are shown only by $V^{IV}O$ compounds and not by 'bare' V^{IV} species.

The possibility that the transformation of a non-oxido to an oxido complex is necessary for a vanadium compound to exhibit insulin-mimetic activity has been considered in the literature. Previous studies by the Crans/Willsky team recognized that the redox properties of the V complexes show a strong correlation with their effectiveness in the animal models of diabetes.[39] Based on the observation that all the V species with anti-diabetic activity undergo irreversible aqueous redox chemistry as determined by cyclic voltammetry, they examined the hypothesis that a complex with reversible redox chemistry would not be effective as an insulin-mimetic agent *in vivo*. Therefore, the Crans/Willsky team tested amavadin-like† non-oxido V^{IV} complexes; the results in streptozotocin (STZ)-induced diabetic Wistar rats demonstrated that this class of compounds did not show any insulin-like effects.[39] The redox potentials and reversibility for the amavadin-like† V^{IV} species are well established in the literature,[40] whereas those of the non-oxido V^{IV} species described here are tabulated in Table 3.

Table 3 Redox potentials and type of process for the $V^{IV}/V^{IV}O$ species studied in this work.

Ligand	Solvent	Couple	Process	$E_{1/2}$	Ref.
taci	H_2O	V^{IV}/V^{III}	Reversible	0.00 V [a]	20
taci	H_2O	V^{V}/V^{IV}	Reversible	+1.15 V [a]	20
ino	H_2O	V^{IV}/V^{III}	Reversible	-0.64 V [a]	21
ino	H_2O	V^{V}/V^{IV}	Quasi-reversible	+0.64 V [a]	21
H_2dhab	CH_2Cl_2	V^{IV}/V^{III}	Reversible	-0.03 V [b]	25
H_2dhab	CH_2Cl_2	V^{V}/V^{IV}	Reversible	+1.16 V [b]	25
H_4anth	H_2O	V^{IV}/V^{III}	Reversible	-0.89 V [b]	*c*
H_2hyph^{Ph}	H_2O/MeOH	$V^{IV}O/V^{III}$	Irreversible	-0.18 V [b]	*c*
H_2cat	H_2O	$V^{IV}O/V^{III}$	Irreversible / reversible[d]	-0.72 V [e]	23
H_2pdbh	CH_2Cl_2	V^{IV}/V^{III}	Reversible	-0.54 V [b]	25
H_2pdbh	CH_2Cl_2	V^{V}/V^{IV}	Reversible	+1.10 V [b]	25
H_2pdbh	H_2O	$V^{IV}O/V^{III}$	Irreversible	-0.46 V [b]	*c*

[a] *vs.* NHE. [b] *vs.* Ag/AgCl electrode. [c] This work. [d] Irreversible when low ligand to metal ratio are used (presence in solution of $V^{IV}O$ species), reversible for a ligand excess (presence of non-oxido V^{IV} species). [e] *vs.* SCE.

For $[V(taci)_2]^{4+}$ and $[V(inoH_{-3})_2]^{2-}$ the reduction and oxidation to the corresponding V^{III} and V^{V} state is reversible or quasi-reversible.[20-21] This behaviour is explainable only if the coordination geometry and the metal environment remains the same as the non-oxido form in the redox process. Cyclic voltammograms recorded in the $V^{IV}O^{2+}/H_2$cat system in aqueous solution show that the reduction to V^{III} is reversible when the main species in solution is the six-coordinated complex $[V^{IV}(cat)_3]^{3-}$ which forms in the presence of an excess ligand;[23] however, it becomes irreversible when low ligand to metal molar ratios are used and $V^{IV}O$ species exists in solution.[23] The behaviour of $[V(dhab)_2]$ and $[V(pdbh)_2]$ differ

from that of the complexes described above and is worth being discussed. In a non-coordinating solvent, such as CH_2Cl_2, they maintain their structure (as confirmed by EPR measurements[18]) and the reduction to V^{III} is reversible.[25] In an aqueous solution $[V(anth)_2]^{4-}$ (formed by H_4anth, a H_2dhab derivative,[18] see Scheme 2) keeps its hexa-coordinated structure and the reduction to $[V^{III}(anth)_2]^{5-}$ is reversible; in contrast, in water $[V(pdbh)_2]$ transforms into $[VO(pdbh)_2H]^-$ (Scheme 3) and the reduction to the corresponding V^{III} species becomes irreversible. Therefore, when a non-oxido V^{IV} species transforms into an oxido $V^{IV}O$ species, the reduction to V^{III} is irreversible because the process entails the loss of the oxido ligand.[41]

We hypothesize here that the anti-diabetic activity and mode of action of V compounds can be related to the structural features of the coordination complex, specifically the presence of the V≡O unit. We observe that the oxido species are pharmacologically active, whereas the non-oxido species are inactive. Therefore, the type of redox process (reversible or irreversible), observed by the Crans/Willsky team,[39] is an effect due to the structural features of the specific V compound (non-oxido or oxido) and not the cause of its anti-diabetic activity. We propose that the lack of insulin-enhancing properties of amavadin derivatives† can be attributed to the fact that they are non-oxido complexes at physiological pH.[39] No transformations into the oxido species were found to take place for these compounds.[39] Stated generally, this observation is a direct consequence of a ligand that complexes the metal ion so strongly that no other species form under the biological conditions. In the case of the amavadine derivatives†, then non-oxido V^{IV} compounds prevail and the V≡O unit, important for processing into the bio-active forms, is lacking. Based on these considerations, we suggest that the formation of the V≡O group is more important for the insulin-mimetic action than the nature of the species (a $V^{IV}O$ compound is active, a 'bare' V^{IV} compound is not active).

These considerations also apply to any anti-diabetic activity of vanadium(III) compounds. Although V^{III} species generally does not contain an oxido group, the structural observations reported here can be extended to the complexes in this oxidation state. V^{III} compounds have not been extensively explored as potential insulin-enhancing agents as V^{IV} and V^{V} species and only a few examples of anti-diabetic V^{III} coordination complexes have been reported. These include V^{III} species of maltol, hydroxypyrones and hydroxypyridinones, which are less studied than $V^{IV}O$ and $V^{V}O$ species because of their tendency to oxidize and undergo hydrolytic processes at pH 7.4.[16] In this context, the analysis of the series of V complexes across oxidation states formed by 2,6-pyridinedicarboxylic acid (H_2dipic), $[V^{III}(dipic)_2]^-$, $[V^{IV}O(dipic)(H_2O)_2]$ and $[V^{V}O_2(dipic)]^-$, allows direct comparison.[17] Among the three oxidation state, those of $V^{IV}O$ and V^{V} are the most effective as anti-diabetic agents. The different activity indicates that the redox processes should be important for the compounds' pharmacological action and the authors suggested that the effectiveness of V compounds may depend both on the ligand and metal oxidation state.[17] These results were further substantiated with a corresponding study on the chloro substituted dipicolinate.[14] On the basis of the results of this study, we hypothesize that the lower insulin-mimetic activity of V^{III} complexes is due to lacking the V≡O chemistry. Although the V^{III} compounds are likely to convert to $V^{IV}O$ and $V^{V}O/V^{V}O_2$ systems at physiological conditions, the resulting pharmacological activity will be less than that of a V^{IV} or V^{V} species.

Finally, redox processes take place *in vivo* between V^{IV} to V^{V} species and must be considered because similar conversions of $V^{V}O/V^{V}O_2$ may be important to the suggestions proposed here. X-ray absorption spectroscopic studies have shown that, in a biological system, both V^{IV} to V^{V} are present in the cells treated with $V^{IV}O$ complexes.[42] Other studies have proved the formation of V^{IV} after addition of V^{V} in several biosystems.[13, 43, 44] Even if a redox cycle has not been demonstrated and the relative amount of V^{IV} and V^{V} species is not known, an equilibrium between the two oxidation states in the cellular environment can be established.[5, 44-45] Therefore, both $V^{V}O/V^{V}O_2$ and $V^{IV}O$ may contribute to the insulin-like action. It is possible that such contributions are synergistic, but also that one oxidation state (such as V^{IV}) may be more important, if the other ones (such as V^{V}) were only precursors or partially involved. Consideration of this question is beyond the scope of this work and needs investigation of additional compounds containing V^{V}.

4. Conclusions

We have tested a series of non-oxido V^{IV} compounds that, after forming a V≡O moiety, exhibit anti-diabetic effects and we have shown that the formation of $V^{IV}O$ species is necessary to their insulin-mimetic action. Currently, these anti-diabetic effects are generally attributed to the inhibition of regulative protein tyrosine phosphatases (PTPs).[5, 11b] In fact, the species with V≡O bonds are found to be more potent inhibitors for phosphatases than the V^{III} and V^{IV} compounds with 'bare', hexa-coordinate geometry.[40b] The current prevailing opinion is that the V complexes are sources of an uncomplexed form of vanadium, which is causing the insulin-enhancing effects. It was proposed – and recently re-affirmed – that, because at about neutral pH V^{IV} exists as $[V^{IV}O(OH)_3]^-$, this may be the inhibiting species.[1f, 11b, 46] This proposal is consistent with the observation that aqueous V^{IV} is a potent inhibitor of several phosphatases, in some cases as effective as V^{V}.[11b, 47] Therefore, it is possible that the effects of $[V^{IV}O(OH)_3]^-$ (= $H_3V^{IV}O_4^-$) may contribute to those of the structurally similar species of V^{V}, $[V^{V}O(OH)_3]$ (= $H_3V^{V}O_4$). It is very difficult to provide unambiguous evidence for biotransformations of a particular V complex in the organism because of the complexities of the biological systems and, therefore, it is not possible to establish which of the two oxidation states is active *in vivo*; the best that can be done is the recognition of the pharmacological effects.

In this study, we have used the combination of thermodynamic, spectroscopic and electrochemical data to relate the pharmacological action of V^{IV} compounds to the biotransformation of such species under the physiological conditions. Other modes of action of V complexes exist, including affecting the signal transduction through interaction with the cellular membrane or altering the redox environment of the cell. However, the hypothesis presented here that 'bare' V centers do not exert the insulin-enhancing effects is novel and can, at least in part, explain why the active V species have remained elusive for so long.[1]

Notes and references

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† Amavadin is a non-oxido V^{IV} complex found in the several mushrooms of genus *Amanita*, formed by the bioligand *N*-hydroxyimino-2,2'-diisopropionic acid in its trianionic form (see refs. [5] and [11b]).

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