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INSULIN-MIMETIC AND SPECIATION STUDIES OF OXIDOVANADIUM COMPLEXES CONTAINING PICOLYLTRYPTOPHANE AND *TRIS*(PYRIDYL)AMINE

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Abstract

The new oxidovanadium complexes $[V^{IV}O(pic\text{-}trpMe)_2]$ (**1**) and $[V^{V}O(pic\text{-}trpH)tpa]^{2+}$ (**2**), where pic-trpMe = *bis*(pyridine-5-L-tryptophanmethylester-2-carboxylate and tpa = *tris*(methyl pyridyl)amine, have been synthesised and characterised. The speciation in the systems VO^{2+}/picH-trpH has been revealed for the pH range 3-8, showing that the dominating complexes in aqueous

solution are $[VO(pic\text{-}trpH)(pic\text{-}trp)]^-$ (pH 3-5.5) and $[VO(pic\text{-}trp)(OH)]^-$ (pH 5-7). The inhibition of lipolysis, one of the functions of insulin, has been studied *in vitro*, using rat adipocytes. Complex **2** and $[VO(tpa)SO_4]$ **3**, and also picH-tprMe show some activity (**2** > $[VO(tpa)SO_4]$ > picH-tprMe), although not to the extent of $[VOSO_4]$ (IC_{50} = 0.81(20), **2**: 1.84(40)).

Keywords: vanadium, oxidovanadium, picolinate, speciation, insulin-mimetic.

Resumen

Se sintetizaron y caracterizaron los nuevos complejos $V^{IV}O(pic\text{-}trpMe)_2]$ (**1**) y $[V^{V}O(pic\text{-}trpH)tpa]^{2+}$ (**2**), donde pic-trpMe = *bis*(piridina-5-L-triptofaneamidametilester-2-carboxilato y tpa = *tris*(metillpiridil)amina. La especiación en los sistemas VO^{2+}/picH-trpH se reveló para el rango de pH 3-8 mostrando que los complejos dominantes en solución acuosa son $[VO(pic\text{-}trpH)(pic\text{-}trp)]^-$ (pH 3-5.5) y $[VO(pic\text{-}trp)(OH)]^-$ (pH 5-7). La inhibición de la lipólisis, una de las funciones de la insulina, se ha estudiado *in vitro* usando rat adipocitos. Los complejos **2** y $[VO(tpa)SO_4]$ **3**, y también picH-tprMe mostraron alguna actividad (**2** > $[VO(tpa)SO_4]$ > picH-tprMe), aunque no en la extensión de $[VOSO_4]$ (IC_{50} = 0.81(20), **2**: 1.84(40)).

Palabras clave: vanadio, óxido vanadio, picolinato, especiación, insulino-mimético.

Introduction

The similarity between vanadate and phosphate results in a well established phosphate-vanadate antagonism [1, 2], leading to inference of vanadate with many phosphate-dependent and phosphate-metabolising enzymes, commonly an inhibitory effect. The inhibition of a cytosolic proteintyrosinephosphatase (PTPase) by vanadate has been proposed to be a key step in the insulin-mimetic/enhancing effect of many vanadium compounds [3, 4], and may thus be responsible for the potential of vanadium compounds in the treatment of diabetes mellitus type 1 and type 2. Inhibition of PTPase prevents the hydrolysis of tyrosinephosphatase at the cytosolic site of the insulin receptor, and thus helps keeping intact the signal transduction pathway for the activation of the glucose carrier Glut4 [2], responsible for the cellular glucose uptake otherwise triggered by insulin. Apart from this specific role of insulin (and vanadate) in lowering the blood glucose level, its role in the inhibition of lipolysis (and the stimulation of lipogenesis) is noteworthy, a function which can also be effected by vanadium compounds [5].

The first vanadium compounds to be tested with respect to their ability to suppress the appearance of diabetes mellitus were inorganic in nature, such as vanadate [6, 7] (which tends to be toxic), peroxovanadates [8] (which tend to exert oxidative stress), and vanadylsulphate $[VOSO_4]$ [9] (which is poorly absorbed in the gastro-intestinal tract). Organic vanadium compounds, i.e. coordination compounds carrying organic ligands, can combine high efficacy with low toxity, the high efficacy being due to effective absorption, and effective uptake by the target cells. Among these ligand systems are maltol [10], picolinate [11] and derivatives of picolinic acid, such as 5-alkoxocarbonyl-picolinates which, as we have shown recently [12], can be operative in stimulating cellular glucose uptake and inhibiting lipolysis *in vitro*, if the alkyl substituents are chosen so as to provide a balanced lipo-/hydrophilicity, or are recognised by cell membrane receptors (such as the galactosyl substituents). Here, we wish to report on initial results obtained with 5-amide derivatives of 2,5-dipicolinic acid (where the amide function is provided by the amino acid L-tryptophan-methylester, viz. picH-trpMe) in the presence of a neutral, potentially tetradentate co-ligand, *tris*(methylpyridyl)amine, tpa, an oxidovanadium(IV) complex of which, $[VO(tpa)SO_4]$, has recently been characterised [13]. For the ligands employed, cf. Fig. 1.

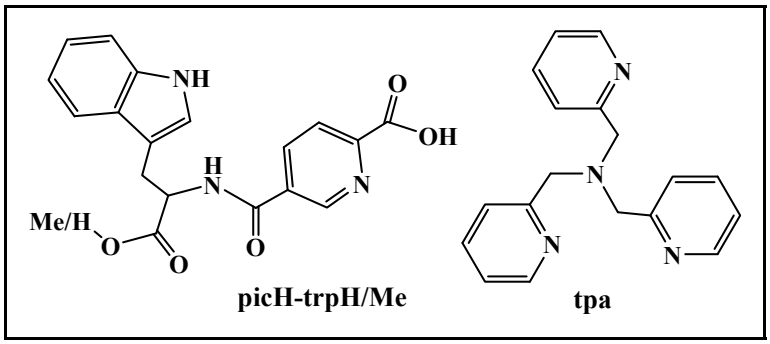

Figure 1. Ligands employed in the present study.

Results and Discussion

The synthesis of the protected proligand, the dimethyl ester picMe-trpMe, follows the strategy described previously for the esters of 5-alkoxocarbonyl-picolinic acid [12]: Starting from 1,5-dipicolinic acid, 2-methoxycarbonyl-5-C(O)Cl-picolinate is generated, and reacted with tryptophan-methylester to yield picMe-trpMe in 82% yield. The IR spectrum shows characteristic bands at 1738 C(O)OMe, 1657 ν(CO), and 1531 cm^{-1} C(O)NH. The compound was further characterised by its ^{1}H-^{13}C HSQC NMR and FAB-MS (*m/z* = 328). For the preparation of the V^{IV} complex [VO(pic-trpMe)$_2$] **1** (Fig. 2), vanadylsulphate pentahydrate and the pre-ligand picMe-trpMe (VO^{2+}:ligand = 1:2) were refluxed in THF/H_2O plus added sodiumacetate to yield a green solution, from which **1** was isolated by precipitation from the concentrated solution, followed by recrystallisation, in yields of 34%.

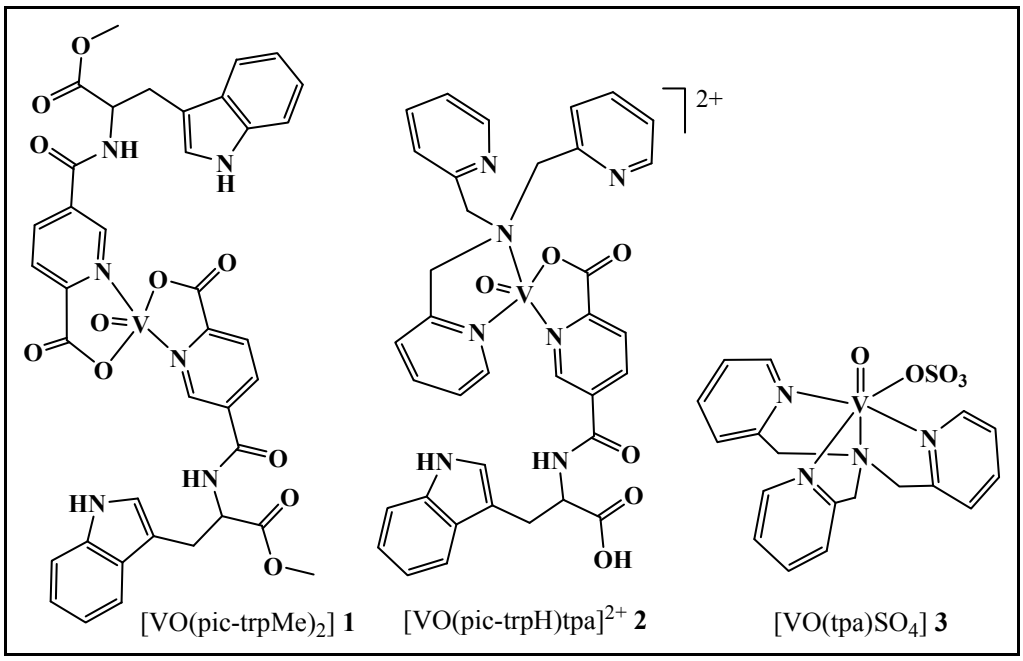

Figure 2. Complexes synthesised and employed in the present study. For **1** and **2**, the possible solution structures are shown (based on EPR in case of **1** and ^{51}V NMR in case of **2**). For **3**, see crystal structure results [13].

The ν(V=O) of **1**, 975 cm^{-1}, is in the typical range for oxidovanadium(IV) complexes where the oxido ligand is not additionally involved in hydrogen bonding or V=O···V interactions. The coordination mode of the ligand proposed for **1** in Fig. 1 is based on EPR results in frozen methanol. Under these anisotropic conditions, the complex shows a 16-line pattern typical of square-pyramidal VO^{2+} complexes with negligible trigonal distortion; Table 1: The *z* component of the anisotropic hyperfine coupling constant, A_z, , amounts to $A_z = 166 \cdot 10^{-4}$ cm^{-1}. The A_z for an equatorial ligand set ($O_{carboxylate}$, $O_{carboxylate}$, N_{pyr}, N_{pyr}), calculated on the basis of the additivity relationship $A_z = \Sigma a_i$, where the a_i are the partial ligand contributions and summation is carried out over the 4 ligands in the equatorial plane [14, 15], is $A_z = 165 \cdot 10^{-4}$ cm^{-1}. Participation of the indole-N or amide-N in coordination would require deprotonation of the respective functions, which would result in a clearly smaller A_z, while replacement of coordinated pyridine by additional oxo-functional groups (like methanol or Trp-carboxylate after deprotection) would *in*crease A_z.

Table 1. EPR data for **1**; *A* in units of 10^{-4} cm^{-1}.

g_{iso}	A_{iso}	$g_{x,y}$	g_z	A_{xy}	A_z
1.968	96	1.980	1.945	62	166

The cationic oxidovanadium(V) complex **2** was prepared by treatment of a 1:1 mixture of $VO(OiPr)_3$ and tpa·HCl dissolved in methanol/THF (2:5) with an equimolar amount of a methanolic solution of picH-trpH (obtained by deprotection of picH-trpMe with KOH, followed by treatment with HCl and extraction with $CHCl_3$), and recrystallisation from methanol/toluene to yield **2**Cl_2·0.8toluene (composition based on elemental analysis). Characteristics: $\delta(^{51}V)$ ($CDCl_3$/MeOH 3:1) = -485 ppm; selected IR bands: 1713 ν(C=O); 1608, 1573 $\nu_{as}(CO_2^-)$; 1380, 1351 ν(C=N) and $\nu_s(CO_2^-)$; 978 ν(V=O). The structure proposed in Fig. 2 takes into account that the $\delta(^{51}V)$ value reflects a donor set dominated by N-functional ligands and excluding directly coordinated Cl^- [16].

In order to study the stability of complex **1** in the pH range relevant under physiological conditions, speciation studies on the binary system VO^{2+}/AH_2 (AH_2 ≡ picH-trpH) were carried out in the pH range 3-8, that is a pH range covering the situation in the stomach (about pH 2-3), the small intestines (slightly alkaline) and blood plasma (pH 7.35). The results are summarised in Table 2 and Fig. 3. The constants listed in Table 2 are defined as follows:

Protonation constants of the ligand: $\log\beta(AH_2)$: $AH_2 \leftrightarrows AH + H^+$; $\log\beta(AH_1)$: $AH_1 \leftrightarrows A + H^+$
Protonation constants of the complexes: $\log\beta(VOA_nH_x)$: $VOA_nH_x \leftrightarrows VOA_nH_{x-1} + H^+$
Stability constants of the complexes: $pK(VOAH_x/VOAH_{x-1})$: $VO^{2+} + AH_x \leftrightarrows VOAH_{x-1} + H^+$

As can be seen on inspection of Fig. 3, the *bis*(ligand) complex $[VOA_2H]^-$ ≡ [VO(pic-tprH)(pic-tpr)]$^-$ (containing one of the ligands in the mono- and one in the di-deprotonated form) is stable in the acidic range only. The slightly acidic to neutral range is dominated by the *mono*(ligand) complex $[VOAH_{-1}]^-$ ≡ $[VO(pic\text{-}trp)(OH)(H_2O)_x]^-$ while, above pH ≈ 7.5, only hydroxidovanadates devoid of any ligand exist. As has been shown previously for related ligands, the *mono*(ligand) complexes can be stabilised clearly beyond pH 7.5 in the presence of a third

component, such as the low-molecular mass blood constituents citrate, phosphate or lactate by formation of ternary complexes of the general composition [VO-A-B] [12a, 17].

Table 2. Ligand protonation constants $\log\beta(AH_x)$ and acid constants pK_a, and complex protonation constants $\log\beta(VOA_nH_x)$ and stability constants $\log K(VOAH_x/VOA)$ in aqueous solution at an ionic strength of 0.2 M (KCl), T = 25 °C.

$\log\beta(AH_2)$[a] pK_{a1}	$\log\beta(AH_1)$ pK_{a2}	$\log\beta$(VOAH) pK(VOAH/VOA)	$\log\beta$(VOA) pK(VOA/VOAH$_{-1}$)	$\log\beta$(VOAH$_{-1}$)	$\log\beta$(VOA$_2$H)
3.89(1) 2.86	6.75(1) 3.89	9.3(2) 3.99	5.31(11) 5.45	-0.12(6)	13.57(9)

[a]Mean deviation in parentheses.

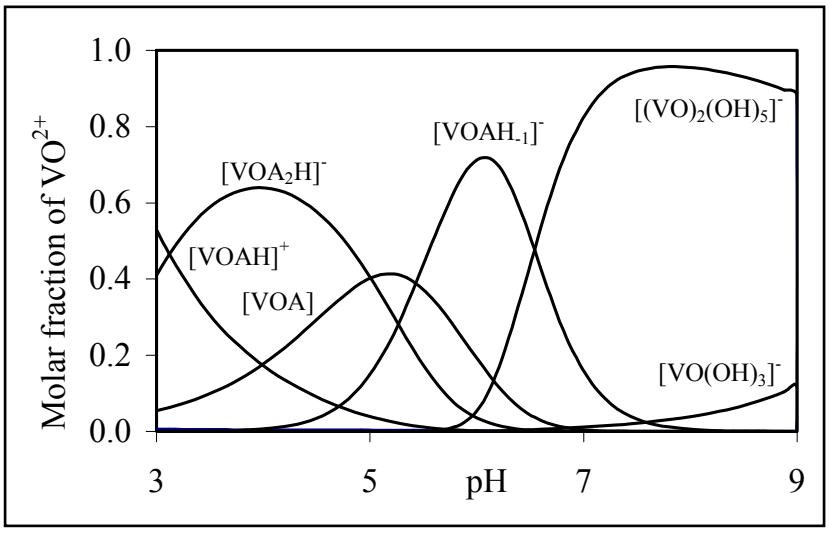

Figure 3. Speciation diagram for the system VO^{2+}/AH_2 (AH_2 = picH-trpH); $c(VO^{2+})$ = 1 mM, $c(AH_2)$ = 2 mM.

Since, based on the above speciation studies, complex **1** does not appear to be sufficiently stable at neutral pH, we have employed the ternary complex **2** along with [VO(tpa)SO_4] (**3**), picH-tprMe, and [$VOSO_4$] as a bench mark compound to study the effect of the vanadium complexes on the inhibition of lipolysis (suppression of the hydrolysis of triglycerides). For these experiments (for details on the assay employed see ref. [12]), freshly prepared adipocytes from male Wistar rats were incubated (37 °C, 30 min) with solutions of the vanadium complexes at three concentrations (0.1, 0.5 and 1 mM in isotonic saline + 5 mM glucose), followed by 180 min incubation with epinephrine (= adrenaline, 10 μM), an antagonist of insulin. The inhibitory effect was determined in terms of the decrease of the amount of free fatty acids (FFA) released by the adipocytes. The results are depicted in Fig. 4. Table 3 summarises the IC_{50} values, which are defined as the concentrations c(V) at which 50% inhibition of FFA release takes place.

Inspection of Fig. 4 reveals high FFA for the cells treated with the insulin antagonist (epi). The FFA level clearly goes down in the presence of 0.5-1 M vanadylsulphate (VO^{2+}), which is

present in solution mainly in the form of $[VO(H_2O)_4OH]^+$. The ternary complex **2** is also effective at *c*(V) = 1 mM, although not to the extent as $VOSO_4$. The binary complex **3** is even less efficient, which may be due to very tight coordination of the tetradentate N-functional ligand, preventing efficient cellular uptake or, if taken up into the cytosol, insufficient degradation to provide active vanadium species. Interestingly, the ligand picH-trpMe also shows activity.

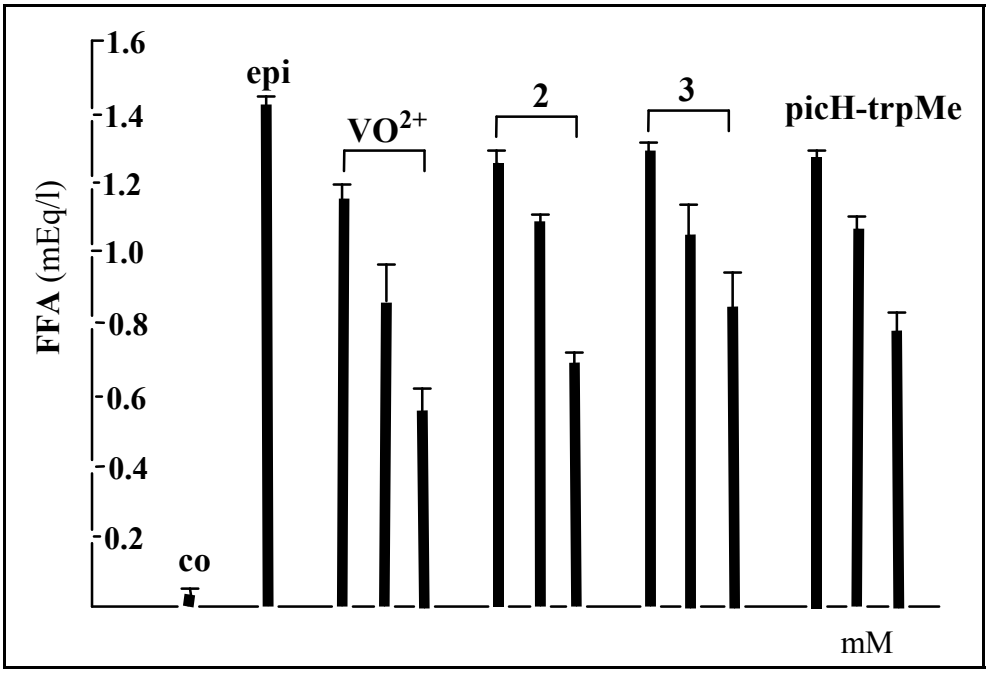

Figure 4. Insulin-mimetic action of vanadium complexes and picH-trpMe on the inhibition of lipolysis in rat adipocytes. Abbreviations: FFA = free fatty acids, co = control, epi = epinephrine, VO^{2+} = vanadylsulphate; for the ligand picH-trpMe and the complexes **2** and **3**, see Figs. 1 and 2, respectively. The T-shapes on top of the bars indicate mean deviations. The concentrations for the triplets of bars are (from left to right) 0.1, 0.5 and 1 mM.

Table 3. IC_{50} (mM) values calculated from the data in Fig. 4.

$VOSO_4$	**2**	**3**	**picH-trpMe**	**tpa**
0.81 ± 0.2	1.84 ± 0.4	4.60 ± 0.7	7.8 ± 3.9	none

In conclusion, we have shown that a binary *bis*(ligand) complex (**1**) forms between VO^{2+} and tryptophan-derivatised 2,5-dipicolinate (pic-trpMe) with, in methanol solution, an equatorial N_2O_2 donor set provided by the two 2-picolinate moieties. In *aqueous* solution, the composition depends on the pH, the two main species being a *bis*(ligand) (pH optimum ca. 4) and a *mono*(ligand) complex (pH optimum ca. 6.5). At the pH pertinent to blood (7.35), this complex is not likely to survive. The more stable ternary, cationic complex **2**, formed between VO^{3+}, pic-trpH

and *tris*(methylpyridyl)amine (tpa) shows *in vitro* (rat adipocytes) insulin-mimetic activity with respect to the inhibition of lipolysis at concentrations > ca. 0.5 mM.

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