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Spectroscopic Characterization of an Oxovanadium(IV) Complex of Oxodiacetic Acid and 2,2'-Bipyridine. Bioactivity on Osteoblast-Like Cells in Culture

Ignacio E. León,[1,2] Susana B. Etcheverry,[1,2] Beatriz S. Parajón-Costa,[2] and Enrique J. Baran [*2]

[1] Cátedra de Bioquímica Patológica, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 1900 La Plata, Argentina.

[2] Centro de Química Inorgánica (CEQUINOR/CONICET,UNLP), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, C. Correo 962, 1900 La Plata, Argentina.
(+54)2214259485 baran@quimica.unlp.edu.ar

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Abstract. The oxovanadium(IV) complex of oxodiacetic acid (H_2ODA) and 2,2'-bipyridine (bipy) of stoichiometry $[VO(ODA)(bipy)]\cdot H_2O$, was thoroughly characterized by infrared, Raman and electronic spectroscopies. The biological activity of the complex on the cell proliferation was tested on osteoblast-like cells (MC3T3E1 osteoblastic mouse calvaria-derived cells and UMR106 rat osteosarcoma-derived cells) in culture. The complex caused inhibition of cellular proliferation in both osteoblast-like cells in culture, but its action was statistically stronger in the tumoral cells. This effect was specially marked with increasing concentrations of the complex. Based on these preliminary biological results, $[VO(ODA)(bipy)]\cdot H_2O$ can be considered as a good candidate to be further investigated in relation to cancer treatment.
Keywords: Oxovanadium(IV), oxodiacetic acid, 2,2'-bipyridine, vibrational spectra, electronic spectra, osteoblast cells in culture, antitumoral action.

Resumen. El complejo de oxovanadio(IV) del ácido oxodiacético (H_2ODA) y 2,2'-dipiridina (bipy) de estequiometría $[VO(ODA)(bipy)]\cdot H_2O$, fue caracterizado detalladamente por espectroscopia infrarroja, Raman y electrónica. La actividad biológica del complejo sobre la proliferación celular fue ensayada sobre células osteoblásticas de fenotipo normal (MC3T3-E1) y tumorales (UMR106) en cultivo. El complejo fue más deletéreo sobre los osteoblastos tumorales que sobre los de fenotipo normal, siendo este efecto más marcado con el aumento de la concentración del mismo. Los resultados de estos estudios biológicos permiten considerar al $[VO(ODA)(bipy)]\cdot H_2O$ como un buen candidato para futuros estudios en relación a los tratamientos antitumorales.
Palabras clave: Oxovanadio(IV), ácido oxodiacético, 2,2'-dipiridina, espectros vibracionales, espectros electrónicos, osteoblastos en cultivo, acción antitumoral.

Introduction

The biodistribution, toxicology and biological effects of vanadium, as well as its pharmacological activity, are areas of increasing research interest [1,2]. The potentiality of simple vanadium compounds and complexes as therapeutic agents has been repeatedly emphasized in recent years and its insulin-mimetic [1-6], anti-tumoral [2,7-10], anti-parasitic [11] and anti-microbial activities [12] have been reported.

Although information about the metabolism of physiologically amounts of vanadium in the higher forms of life, including man, remains scarce, some general aspects related to the absorption, transport, biological transformations, toxicity, excretion and accumulation of vanadium are relatively well-known [1,13,14] In this context the fact that bone seems to be the major sink for retained vanadium has been unambiguously demonstrated by numerous studies [13-15]. This fact determines the great interest to investigate the effects of vanadium compounds on osteoblast-like cells in culture.

As part of a research project devoted to the search and characterization of new vanadium complexes with pharmacological activity, we have now investigated the spectroscopic behavior and the biological effects of a new oxovanadium(IV) complex of stoichiometry $[VO(ODA)(bipy)]\cdot H_2O$ with ODA = anion of the oxodiacetic acid (H_2ODA = $O(CH_2COOH)_2$) and bipy = 2,2'-bipyridine [16], on the proliferation of two osteoblastic cell lines, one normal (MC3T3-E1) and the other tumoral (UMR106).

Results and discussion

Structural characteristics of $[VO(ODA)(bipy)]\cdot H_2O$

The crystal structure of the complex was determined by del Río *et al.* using single crystal X-ray diffractometry [16]. As shown in Figure 1, the coordination geometry around the metal center is a distorted octahedron. The basal plane is generated by the two bipyridine N-atoms and two carboxylic O-atoms from the acetate groups. The axial positions are occupied by the central oxygen atom of ODA and the oxo O-atom of the V=O group [16]. The shortest V-O distances correspond to the V=O bond and the V-O_{ether} bond is somewhat longer than those to the carboxy-oxygens. The crystallization water molecule is involved in hydrogen bonds between this molecule and uncoordinated carboxylate oxygen atoms [16].

Vibrational spectrum of $[VO(ODA)(bipy)]\cdot H_2O$

In order to analyze the IR and Raman spectra of the complex, we have compared these spectra with those of the free oxodiacetic acid [17], 2.2'-bipyridine [18,19] and the recently

investigated and related [VO(ODA)(phen)]·1.5H_2O complex (ophen = 1,10-phenanthroline) [20]. The spectra obtained for the [VO(ODA)(bipy)]·H_2O complex are shown in Figure 2 and the proposed assignment is presented in Table 1. The assignments are additionally supported by information provided by well known standard reference texts [21,22] and are briefly commented, as follows:

- The carboxylate groups show the typical spectroscopic behavior expected from a monodentate interaction of this moiety with the metallic center, i.e., the antisymmetric stretching vibration presents a lower energy than those found for the ν(C=O) vibration in the "free" acid (1734 cm^{-1}), whereas the symmetric mode lies somewhat higher than the corresponding ν(C-O) mode (1309 cm^{-1} in the "free" acid) [17]. Besides, both carboxylate stretchings also show an energy difference (about 260 cm^{-1}, from the IR data) which is characteristic for monodentate binding [23].

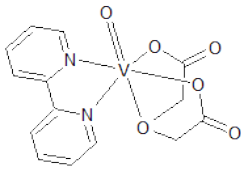

Fig. 1. Schematic structure of the investigated complex, [VO(ODA)(bipy)]·H_2O.

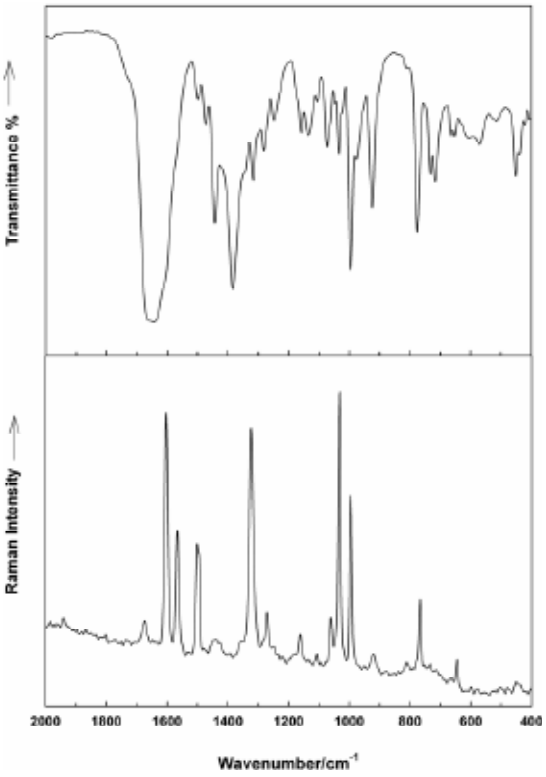

Fig. 2. FTIR (above) and FT-Raman (below) spectra of [VO(ODA)(bipy)]·H_2O in the spectral range between 2000 and 400 cm^{-1}.

Table 1. Assignment of the vibrational spectra of [VO(ODA)(bipy)]·H_2O (band positions in cm^{-1})

IR	Raman	Assignments
3438 vs,br		ν(OH) (H_2O)
3116 w, 3086 w, 3033 vw	3082 m	bipy - ν(CH)
2988 vw, 2931w	2985 w, 2933 m	$\nu(CH_2)$
1643 vs	1670 w	$\nu_{as}(COO^-)$
	1605 vs, 1567 s	bipy - ν_{ring}
1498 w, 1473 w	1502 s	bipy - ν_{ring}
1443 m	1442 w	bipy - ν_{ring} + δ_{ring-H}
1384 vs	1324 vs	$\nu_s(COO^-)$
1316 m		ν_{ring} + inter ring stretch
1282 w, 1247 w	1273 w	bipy - $\delta(CH)_{in\ plane}$
1158 m	1162 w	bipy - ν_{ring} + $\delta(CH)_{in\ plane}$
1135 m, 1106 w	1110 vw	ν_{as}(C-O-C)
1072 m, 1034 m	1061 w, 1032 vs	bipy - $\delta(CH)_{in\ plane}$
995 vs	996 vs	ν(V=O)
976 vw, 923 s	920 w	ν(C-C)
805 sh	812 w	ν_s(C-O-C)
776 s, 733 m, 716 m	758 s	bipy - $\delta(CH)_{out\ of\ plane}$
664 w, 652 w	646 w	$\rho(CH_2)$
616 sh, 570 w		bipy - δ_{ring}
515 w	512 w	δ(C-O-C)
451 s, 422 sh		ν(V-O)

vs, very strong; s, strong; m, medium; w, weak; vw, very weak; w, weak; br, broad; sh, shoulder.

- Regarding the motions of the O_{ether} bond of ODA, both the ν_{as}(C-O-C) and the ν_s(C-O-C) vibrations are slightly displaced to lower energies after interaction with the metal center, whereas the deformational modes are less affected.

- The ν(O-H) water vibrations, centered at about 3400 cm^{-1} in the IR spectrum (not shown in Figure 2), have not Raman counterparts. The corresponding deformational modes are surely overlapped by the strong and broadened $\nu_{as}(COO^-)$ IR-band centered at 1643 cm^{-1}.

- The characteristic ν(V=O) breathing was easy to identify because it generates one of the strongest Raman bands in the spectrum of the complex, and appears in the usually expected region [24] and practically in the same position as in the case of the [VO(ODA)(H_2O)$_2$] [17] and [VO(ODA)(phen)]·1.5H_2O [20] complexes.

- The 2.2'-bipyridine bands were assigned on the basis of previous studies of the free molecule [18,19]. In general, most of the characteristic ring bands in the 1700-1300 cm^{-1} region are slightly displaced to higher frequencies upon coordination and slight displacements and/or intensity changes were also observed in other spectral regions. A similar behavior was

also observed in the case of the *o*-phenanthroline bands in $[VO(ODA)(phen)]\cdot 1.5H_2O$ [20], and it can be considered as characteristic of these type of ligands [25]. Interestingly, some of the bipy-ring motions present very high Raman intensity and have not IR counterparts, whereas the inter-ring stretching vibration could not be identified in the Raman spectrum.

- Ligand-to metal vibrations are difficult to identify. A pair of ν(V-O) stretching modes has been tentatively assigned by comparison with the results obtained in the case of the $[VO(ODA)(H_2O)_2]$ complex [17]. ν(M-N) vibrations involving the nitrogen atoms of 2,2'-bipyridine are usually expected to lie below 300 cm^{-1} [23].

Electronic absorption spectrum of $[VO(ODA)(bipy)]\cdot H_2O$

The electronic absorption spectrum of the complex, measured in a dimethylsulfoxide solution, shows d-d transitions at 650 nm (ε = 13 L/mol.cm), 855 nm (ε = 28 L/mol.cm) and 440 nm (ε = 5 L/mol.cm). This spectrum is in agreement with the simple M.O. model proposed by Ballhausen and Gray for the $[VO(H_2O)_5]^{2+}$ complex [24,26,29]. In this model, the two lower energy bands are assigned to the $b_2 \rightarrow e$ (855 nm) and $b_2 \rightarrow b_1$ (650 nm) transitions, whereas the other one, found at 440 nm is probably the $b_2 \rightarrow a_1$ transition.

In the precursor complex, $[VO(ODA)(H_2O)_2]$, the first two d-d transitions are observed at 792 and 618 nm [17] whereas in $[VO(ODA)(ophen)]\cdot 1.5H_2O$ they are found at 820 and 580 nm [20] . As the 10 Dq parameter can be directly determined from the position of the $b_2 \rightarrow b_1$ transition [26,27], it is clear that the crystal field strength generated in the present case is somewhat weaker to that found in the other two complexes.

Effects of $[VO(ODA)(bipy)]\cdot H_2O$ on the osteoblast-like cell proliferation

Vanadium compounds exert different biological actions *in vitro* and *in vivo* systems depending on several factors not completely understood at present. In fact, the oxidation state and coordination sphere around the metal as well as the nature of the ligands and the cellular types or the *in vivo* models play a complex role in the modulation of vanadium effects [1,28,29].

Figure 3 shows the action of the oxovanadium(IV) complex $[VO(ODA)(bipy)]\cdot H_2O$ on osteoblast proliferation. The effects on two bone related cell lines, one of normal phenotype (MC3T3-E1) and the other tumoral, derived from a rat osteosarcoma (UMR106)), were evaluated by the crystal violet bioassay. As can be seen, the complex inhibited more strongly the proliferation of the osteosarcoma cells than the non-transformed osteoblasts in the whole range of concentration (*$p <$ 0.01). Besides, it can be seen from this figure that in the high concentration range (50 - 100 μM), the complex was markedly more deleterious for the UMR106 cells than for the MC3T3-E1 osteoblasts ($^{\#}p < 0.05$). At the highest tested concentration (100 μM), the MC3T3-E1 cells showed a survival of 56% while for UMR106 it was of 30% (*$p < 0.01$), in comparison with the control condition.

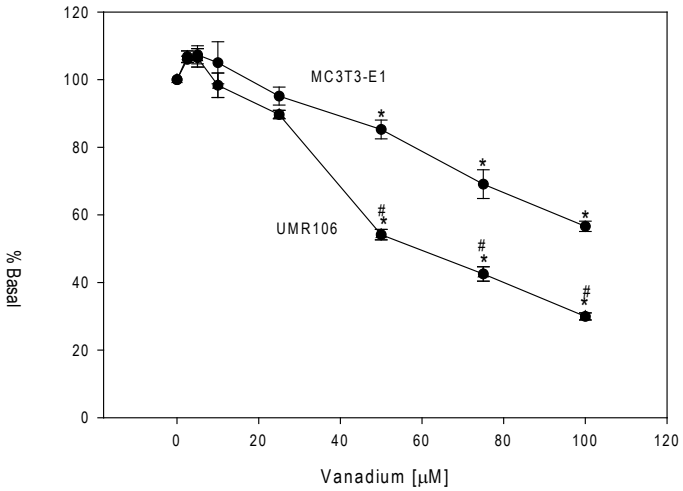

Fig. 3. Effect of $[VO(ODA)(bipy)]\cdot H_2O$ on the osteoblast proliferation. MC3T3-E1 and UMR106 osteoblast-like cells were incubated in serum free DMEM (Basal) or with the addition of different concentrations of the complex for 24 h at 37°C. [Results are expressed as % basal and represent the mean ± SEM, n = 18; *Significant differences vs. control, $p < 0.01$. # Significant differences between samples, $p < 0.05$].

The results of the cell culture study clearly demonstrate the stronger antiproliferative effect of the complex in the osteosarcoma UMR106 cells than in the normal cell line MC3T3-E1.

Comparing with related complexes of the oxovanadium(IV) cation with the multiple oxygen ligand oxodiacetate (ODA), the parent compound of this series, $[VO(ODA)(H_2O)_2]$, was more deleterious in the normal cell line (IC_{50} = 50 μM) than in the tumoral osteoblast cell line (IC_{50} > 100 μM) [17,30] and another ternary complex also investigated by us in osteoblasts in culture, $[VO(ODA)phen]\cdot 1.5H_2O$, showed an IC_{50} of 3 μM for the normal osteoblasts and its IC_{50} value for the UMR106 cells was 17 μM [20]. In the present case, IC_{50} for MC3T3-E1 cells is higher than 100 μM and in UMR106 is 58 μM.

These results are very interesting also from the point of view of vanadium biochemistry, because they show that the replacement of the two water molecules in the parent $[VO(ODA)(H_2O)_2]$ complex by one bidentate ligand produces significant changes in the biological activity of the compound and, even, the replacement by two different (bipy or ophen), but chemically similar, bidentate ligands, produces spectacular changes in the biological assays.

Conclusions

The spectroscopic behavior of the investigated $[VO(ODA)(bipy)]\cdot H_2O$ complex, closely resembled those of the parent $[VO(ODA)(H_2O)_2]$ and of the structurally related $[VO(ODA)phen]\cdot 1.5H_2O$ complexes. The evaluation of the antiproliferative effects of $[VO(ODA)(bipy)]\cdot H_2O$ on two bone related cell lines (one normal and one tumoral), shows that the

complex inhibited more strongly the proliferation of the osteosarcoma cells. Taking into consideration this results and the comparison with the related oxovanadium(IV) complexes, it is clear that the more deleterious action of [VO(ODA)(bipy)]·H_2O on tumoral osteoblasts than on the normal cells, encourage further studies in the field of Medicinal Chemistry exploring the potentiality of this complex for clinical cancer treatments.

Experimental

Materials

Oxovanadium(IV) acetylacetonate were purchased from Fluka, while oxodiacetic acid and 2,2'-bipyridine from Aldrich. Crystal violet, p-nitrophenylphosphate (p-NPP), glycine, $MgCl_2$, and all the other chemicals used in the biological activity experiments were of analytical grade from Sigma Chemical Co. Tissue culture materials were purchased from Trading New Technologies (Buenos Aires, Argentina). Dulbecco's Modified Eagles Medium (DMEM) and fetal bovine serum (FBS) were from GBO-Argentina S.A; trypsin-EDTA was provided by Gibco (Gaithersburg, ND, USA).

Synthesis of the complex

The precursor [VO(ODA)(H_2O)$_2$] was prepared by reaction of oxovanadium(IV) acetylacetonate and oxodiacetic acid, as reported earlier [16,31]. The [VO(ODA)(bipy)]·H_2O complex was obtained by slow addition of 0.176 g (1 mmol) of solid 2-2'-bipyridine to 10 mL of a methanolic solution containing 0.235 g (1 mmol) of the precursor compound, under continuous stirring. The obtained greenish micro crystalline powder was collected by filtration, washed with small portions of cold methanol and dried in air [16]. The purity was confirmed by elemental chemical analysis (Calcd. for $C_{14}H_{14}N_2O_7V$: C, 45.04; H, 3.75; N, 7.51%. Found: C, 45.20; H, 3.78; N, 7.45%).

Fresh stock solutions of the complex were prepared in dimethylsulfoxide at a 100 mM concentration and diluted according to the experimental necessities. The stability of these solutions was confirmed by measuring the variation of the electronic absorption spectra with time.

Spectroscopic characterization

Infrared spectra in the spectral range between 4000 and 400 cm^{-1} were obtained with a Bruker EQUINOX 55 Fourier transform infrared spectroscopy (FTIR) instrument, using the KBr pellet technique. Raman spectra were measured on powdered samples using the FRA 106 Raman accessory of an IF66 Bruker spectrophotometer. Radiation of 1064 nm from a Nd:YAG solid-state laser was used for excitation. Spectral resolution was ± 4 cm^{-1} for both spectral measurements.

Electronic absorption spectra were measured with a Shimadzu model UV-300 spectrophotometer, using 10 mm-quartz cells.

Cell culture and cell proliferation assay

MC3T3-E1 osteoblastic cells derived from mouse calvaria and UMR106 tumoral cells derived from a rat osteosarcoma, were grown in DMEM supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin and 10% (v/v) fetal bovine serum (FBS) at 37 °C, 5% CO_2. When the cells reached 100% confluence, they were sub-cultured using 0.1% trypsin plus 1 mM EDTA in Ca(II)-Mg(II) free phosphate buffered saline (PBS) (11 mM KH_2PO_4, 26 mM Na_2HPO_4, 115 mM NaCl, pH: 7.4) [32,33]. For the experiments, the cells (3×10^4 cells/mL) were grown in 48 well plates during 24 h at 37 °C. Then, the monolayer was incubated with different concentrations of the complex for the proliferation assay.

A mitogenic bioassay was carried out as described by Okajima *et al.* [34], with some modifications. Briefly, cells were grown in forty-eight well plates. After 24 h, they were incubated with different concentrations of the complex (2.5 - 100 μM), according to the caption of Figure 3. Then, the monolayers were washed with PBS buffer and fixed with 5% glutaraldehyde/PBS at room temperature for 10 minutes. After that, they were stained with 0.5% crystal violet / 25% methanol for 10 minutes. The dye solution was discarded and the plate was washed with water and dried. The dye taken up by the cells was extracted using 0.5 ml/well 0.1 M glycine/HCl buffer, pH: 3.0 in 30% methanol and transferred to test tubes. Absorbance was read at 540 nm after a convenient sample dilution. We have previously shown that under these conditions, the colorimetric bioassay strongly correlated with cell proliferation measured by cell counting in Neubauer chamber [32,33].

Statistical methods

At least three independent experiments were performed for each experimental condition. Results are expressed as %Basal and represent the mean ± SEM. Statistical differences were analyzed using the ANOVA test.

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