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The effect of phosphate on the nuclease activity of vanadium compounds

Nataliya Butenko [a,b], José Paulo Pinheiro [a], José Paulo Da Silva [a], Ana Isabel Tomaz [b,c], Isabel Correia [b], Vera Ribeiro [d], João Costa Pessoa [b], Isabel Cavaco [a,b,*]

[a] Departamento de Química e Farmácia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal
[b] Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av Rovisco Pais, 1049-001 Lisboa, Portugal
[c] Faculdade de Ciências da Universidade de Lisboa, Campo Grande, Lisboa, Portugal
[d] Centro de Biomedicina Molecular e Estrutural, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

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ABSTRACT

The nuclease activity of $VO(acac)_2$ (**1**, acac = acetylacetone) and its derivatives $VO(hd)_2$ (**2**, hd = 3,5-heptanedione), $VO(Cl\text{-}acac)_2$ (**3**, Cl-acac = 3-chloro-2,4-pentanedione), $VO(Et\text{-}acac)_2$ (**4**, Et-acac = 3-ethyl-2,4-pentanedione) and $VO(Me\text{-}acac)_2$ (**5**, Me-acac = 3-methyl-2,4-pentanedione), is studied by agarose gel electrophoresis, UV–visible spectroscopy, cyclic and square wave voltammetry and ^{51}V NMR. The mechanism is shown to be oxidative and associated with the formation of reactive oxygen species (ROS). Hydrolytic cleavage of the phosphodiester bond is also promoted by **1**, but at much slower rate which cannot compete with the oxidative mechanism. The generation of ROS is much higher in the presence of phosphate buffer when compared with organic buffers and this was attributed to the formation of a mixed-ligand complex containing phosphate, $(V^{IV}O)(V^{V}O)(acac)_2(H_nPO_4^{n-3})$, presenting a quasi-reversible voltammetric behavior. The formation of this species was further observed by Electrospray Ionization Mass Spectrometry (ESI-MS). Phosphate being an essential species in most biological media, the importance of the formation of mixed-ligand species in other vanadium systems is emphasized.

1. Introduction

Designing metal complexes that can cleave DNA – inorganic nucleases – is a field of numerous applications, from cancer therapy to DNA manipulation. Some vanadium complexes (VCs), in particular derivatives of oxidovanadium(IV) acetylacetonate, $V^{IV}O(acac)_2$, have been shown to be very efficient inorganic nucleases [1]. $V^{IV}O(acac)_2$ and other VCs have also been extensively studied as potential drugs for the treatment of diabetes mellitus and other diseases [2–6].

The aqueous chemistry of VCs can be very complex particularly in dilute solutions. When dissolved in water a VC can form a variety of different species, which may be essentially different from the original compound. In biological fluids such speciation may be more complex. Identifying the species responsible for DNA cleavage is important both to design more efficient nucleases and to assess eventual toxicity of these compounds. Speciation studies of VCs are typically done at millimolar concentration levels [7–11], much higher than the metal concentrations relevant in biological systems, which are in the micromolar or nanomolar range. The speciation of VCs at such low concentrations is not well known or understood, as they are below the detection limits of the most common analytical techniques in metal analysis such as EPR, NMR, UV–visible (UV–Vis) absorption spectroscopy, circular dichroism and potentiometry.

Phosphate is an important biogenic ligand and one of the relevant constituents of human serum and interstitial fluids. It is involved in an extensive number of biological recognition and bio-catalytic systems [6,12–14]. Its concentration in blood is approximately 1.1 mM [15,16], whereas in working muscle cells it can be as high as 40 mM [17]. In spite of being a weak coordinating ligand, when metal complexes are in the micromolar range of concentration, the phosphate ligand-to-metal ratio can be as high as 20,000. The formation of phosphate complexes and mixed-ligand complexes containing phosphate and the original ligand cannot be overlooked.

The few studies conducted on the speciation of vanadium with phosphate were carried out by the groups of L. Pettersson and T. Kiss. Pettersson et al. studied the speciation of vanadates and H_2O_2 in the presence of phosphate and concluded that peroxovanadate-phosphate complexes are not likely to be formed under human physiological conditions [18]. Kiss et al. determined that ternary complexes with phosphate may be formed in blood at neutral pH with maltonate-, picolinate- and methylpicolinate-$V^{IV}O$-systems [8,9].

When studying the nuclease activity of several VCs, we found that not only $V^{IV}O(acac)_2$ was surprisingly active [19], but also that its activity was much higher in phosphate than in the presence of other buffers. This was attributed to the formation of mixed complexes of phosphate and acac and also to a scavenger effect of organic buffers that masks nuclease activity by radicals.

* Corresponding author at: Departamento de Química e Farmácia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.
E-mail address: icavaco@ualg.pt (I. Cavaco).

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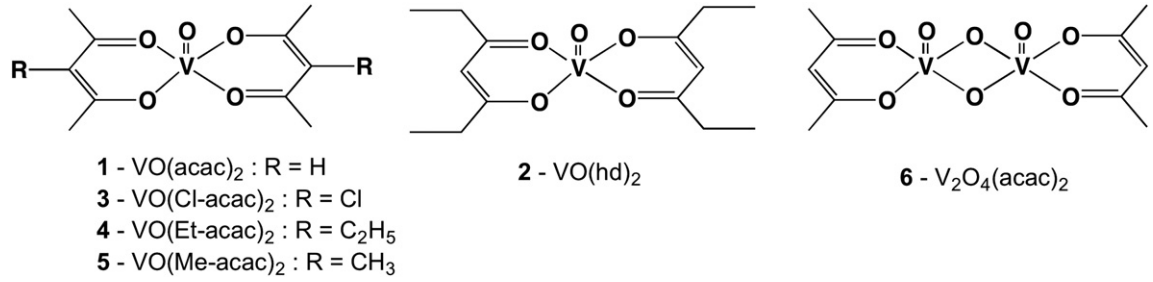

Fig. 1. Proposed structural formulae of complexes **1**–**6**.

The aim of the present work is to understand the nature of DNA cleavage by $V^{IV}O(acac)_2$ derivatives and the role of phosphate in this interaction. Derivatives of $V^{IV}O(acac)_2$ were prepared containing side groups of different nucleophilicity and with different metal oxidation states. Their nuclease activity was assessed by agarose gel electrophoresis (AGE), while solution speciation was evaluated by UV–Vis absorption spectroscopy, ^{51}V NMR spectroscopy, cyclic voltammetry and square wave voltammetry. The nature of the DNA cleavage mechanism – radical or hydrolytic – was assessed by NMR spectroscopy and by spectrofluorometry using well-known probes for this type of reactions.

The complexes studied were $V^{IV}O(acac)_2$ (1, acac = 2,4-pentanedione, also called acetylacetone), $V^{IV}O(hd)_2$ (2, hd = 3,5-heptanedione), $V^{IV}O(Cl\text{-}acac)_2$ (3, Cl-acac = 3-chloro-2,4-pentanedione), $V^{IV}O(Et\text{-}acac)_2$ (4, Et-acac = 3-ethyl-2,4-pentanedione) and $V^{IV}O(Me\text{-}acac)_2$ (5, Me-acac = 3-methyl-2,4-pentanedione). The methyl-, ethyl- and chlorine-groups in 3–5 are substituents of atom C3 of acac and are expected to affect the redox behavior of these complexes. The DNA cleavage activity of a dioxidovanadium(V) complex with acac, $V^{V}{}_2O_4(acac)_2$ (6) and oxidovanadium(IV) sulfate (7) were also studied. The proposed structural formulae of complexes 1–6 are represented in Fig. 1.

2. Experimental

2.1. Materials

All solutions were prepared with Millipore® water of maximum conductivity 0.054 μS/cm. All chemicals used were of analytical grade. $V^{IV}O(acac)_2$ (**1**), $VOSO_4$ (**7**), Hd, Cl-acac, Et-acac, Me-acac, ethidium bromide, trizma® base (≥99.9%), phosphate buffered saline tablets (PBS),[1] agarose powder (type I, low electoendoosmosis), ethidium bromide (EtBr), terephthalate (disodium salt, 98%), 4-nitrophenyl phosphate disodium salt hexahydrate (NPP) and bis-*p*-nitrophenyl phosphate sodium salt (BNPP) were supplied by Sigma-Aldrich; 3-(N-morpholino)propanesulfonic acid (MOPS, 99.5%) was from Fluka; di-potassium hydrogen phosphate (99%) and hydrogen peroxide (30% w/v) were from Panreac; mercaptopropionic acid (MPA, >99%) and potassium peroxomonosulfate (oxone, min 4.5% active oxygen) were purchased from Acros; acetylacetone (99.5%), titriPUR® sodium hydroxide solution and suprapur® and nitric acid (65%) were Merck products; ammonium-*meta*-vanadate was from Riedel-de Haën. Phosphate, HEPES and MOPS buffers (100 mM) were prepared by adjusting the pH to 7.0–7.4 with nitric acid or sodium hydroxide.

Stock solutions of VCs were freshly prepared by dissolving an exact (±0.5 mg) mass of the compound in 200 mL of water or buffer to obtain ~200 μM solution. The natural uncertainty coming from this procedure is ca. 10%. Complex **3** is poorly soluble in water (≪50 μM at room temperature), hence its concentration was unknown. Instead, a saturated solution of **3** was used and diluted to 1:2 and 1:4 to check for concentration effect on nuclease activity. The common procedure of dissolving the complexes in DMSO and diluting with water was not used as DMSO would interfere as a radical scavenger.

2.2. Synthesis

$V^{IV}O(acac)_2$ (**1**), is commercially available and was used as received. Complexes **2**, **4** and **5** were obtained by reaction of $V^{IV}OSO_4$ with an excess of the ligands as previously described [20,21]. Complex **3** was synthesized for the first time following the same procedure. Complex **6** was prepared according to the literature [22]. Fourier transform (FT)-IR spectra in the range 4000–400 cm^{-1} were recorded on a Jasco FT/IR-4100 spectrometer using KBr pellets.

$V^{IV}O(hd)_2$ (**2**): dark green solid; 74.7% yield; elemental analysis (%) calculated for $[VO(C_7H_{11}O_2)_2]\cdot 0.25H_2O$ (found): C 51.62 (51.52); H 6.96 (7.16). IR (KBr, cm^{-1}): 2976, 1552, 1534, 1411, 1371, 1311, 1248, 1186, 1170, 1076, 1000, 991, 954, 861, 810, 779.

$V^{IV}O(Cl\text{-}acac)_2$ (**3**): light green solid; 91.6% yield; elemental analysis (%) calculated for $[VO(C_5H_6O_2Cl)_2]$ (found): C 35.96 (36.01); H 3.62 (3.48). IR (KBr, cm^{-1}): 3001, 2972, 2930, 1576, 1464, 1424, 1374, 1352, 1298, 1049, 1024, 1015, 905, 703, 638, 617, 509, 469, 451.

$V^{IV}O(Et\text{-}acac)_2$ (**4**): green solid; 54% yield; elemental analysis (%): calculated for $[VO(C_7H_{11}O_2)_2]$ (found): C 52.34 (52.19); H 6.90 (7.12). IR (KBr, cm^{-1}): 2962, 2874, 1559, 1467, 1454, 1376, 1331, 1296, 1259, 1173, 1066, 1000, 958, 916, 790, 780, 722, 686, 618, 490, 463, 441.

$V^{IV}O(Me\text{-}acac)_2$ (**5**): light green; 70% yield; elemental analysis (%): calculated for $[VO(C_6H_9O_2)_2]$ (found): C 49.16 (49.05); H 6.19 (6.39). IR (KBr, cm^{-1}): 3015, 2926, 1566, 1480, 1428, 1337, 1300, 1177, 997, 981, 898, 733, 660, 619, 490, 468.

$V_2O_4(acac)_2$ (**6**): dark brown solid; 61% yield; elemental analysis (%): calculated for $[V_2O_4(C_5H_7O_2)_2]\cdot 0.25H_2O$ (found): C 30.67 (30.45); H 3.73 (3.85). IR (KBr, cm^{-1}): 3422, 3112, 1580, 1534, 1413, 1384, 1348, 1294, 1283, 1033, 991, 983, 949, 934, 818, 790, 775, 672, 604.

2.3. Nuclease activity

2.3.1. Agarose gel electrophoresis (AGE)

DNA cleavage activity was tested by AGE using plasmid DNA (pDNA). Commercial pDNA contains EDTA and tris, both of which would interfere in this study [1]. As such, a non-commercial pDNA (pA1) was prepared. pA1 consists of a full-length cDNA from Cytochrome P450 CYP3A1 inserted in the PBS plasmid vector (pBluescribe, Stratagene, UK) [23]. The pDNA was amplified in *Escherichia coli* MACH1 and purified using Nucleobond® AX Anion Exchange Columns for quick purification of nucleic acids from MACHEREY-NAGEL. The concentration of pDNA was measured spectrophotometrically at λ = 260 nm. Each reaction mixture was 20 μL containing 6 μL of water, 2 μL of 100 mM stock buffer solution pH 7.0–7.4, 2 μL of pA1 DNA (0.2 μg) and 10 μL of complex (3–100 μM). The [metal]:[DNA(base pair)] ratio (ri) varied from 0.2 to 6.7. Typically, duplicate controls of supercoiled (Sc) and of linear (Lin) form were introduced into the first

[1] PBS is a pH 7.4 phosphate buffered medium with controlled ionic strength: 10 mM phosphate buffer, 2.7 mM KCl, 137 mM NaCl. When used in the form of tablets higher concentration of pH buffer can be prepared as necessary, keeping the same ratio of its components.