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DNA cleavage activity of $V^{IV}O(acac)_2$ and derivatives

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ABSTRACT

The DNA cleavage activity of several β-diketonate vanadyl complexes is examined. Vanadyl acetylacetonate, $V^{IV}O(acac)_2$, **1**, shows a remarkable activity in degrading plasmid DNA in the absence of any activating agents, air and photoirradiation. The cleaving activity of several related complexes $V^{IV}O(hd)_2$ (**2**, Hhd = 3,5-heptanedione), $V^{IV}O(acac\text{-}NH_2)_2$ (**3**, Hacac-NH_2 = acetoacetamide) and $V^{IV}O(acac\text{-}NMe_2)_2$ (**4**, Hacac-NMe_2 = N,N-dimethylacetoacetamide) is also evaluated. It is shown that **2** exhibits an activity similar to **1**, while **3** and **4** are much less efficient cleaving agents. The different activity of the complexes is related to their stability towards hydrolysis in aqueous solution, which follows the order **1**∼**2** ≫ **3**∼**4**. The nature of the pH buffer was also found to be determinant in the nuclease activity of **1** and **2**. In a phosphate buffered medium DNA cleavage by these agents is much more efficient than in tris, hepes, mes or mops buffers. The reaction seems to take place through a mixed mechanism, involving the formation of reactive oxygen species (ROS), namely OH radicals, and possibly also direct cleavage at phosphodiester linkages induced by the vanadium complexes.

1. Introduction

Some inorganic compounds have demonstrated activity in DNA cleavage and have thus been named *inorganic nucleases*. These compounds are relevant for two main reasons: (a) as possible therapeutic agents in cancer chemotherapy, which is the case of gallium, ruthenium, rhodium, titanium, tin and vanadium compounds [1]; (b) as potential substitutes for natural nuclease enzymes on gene manipulation techniques, for which inorganic nucleases showing site specificity are particularly relevant [2,3].

A different and equally important motivation to study these compounds comes from predicting possible undesirable DNA damage following the use of metal compounds as new options for therapeutical use. Vanadium complexes are well known for their insulin-enhancing properties, and have been studied as possible therapeutic agents for diabetes mellitus since the very discovery of this disease [4]. The complex $V^{IV}O(Et\text{-}maltolato)_2$ has successfully completed phase II of human clinical trials [5,6] and became a benchmark drug candidate in this field. The V^{IV}-complex vanadyl(acetylacetonate), $V^{IV}O(acac)_2$, has been reported to have therapeutic properties, and this has attracted much interest in its biological behaviour [7,8]. It has been proposed as a particularly efficient insulin-enhancing compound [9], and a recent study [10] focused on its anticancer potential and mechanism of action in a human hepatoma cell line, $V^{IV}O(acac)_2$ being found to block the cell cycle permanently at the G1 phase on HepG2 cells.

Among the several transition metal compounds that have shown nuclease activity [11,12], some examples are of vanadium complexes. The first case of nuclease activity by vanadium compounds was reported in 1996 [13] for $VOSO_4$ in the presence of hydrogen peroxide, and explained by Fenton-like generation of hydroxyl radicals. In 2000, several cationic V^{III}-dimeric complexes with 1,10-phenanthroline (phen) and similar ligands were also shown to have nuclease activity [14]. In the same publication, Heater et al. report a strong nuclease activity for the V^{IV}-phen complex. In 2004, V^{IV}-complexes with hydroxysalen ligands showed nuclease activity, in the presence of an activating agent, either a reducing one like mercaptopropionic acid (MPA) or an oxidizing one such as oxone ($KHSO_5$) [15], but the authors did not establish the actual active vanadium species. More recently, several V complexes with photocleavage activity were proposed as promising compounds for cancer phototherapy. The photocleavage activity of phen-peroxovanadate complexes was studied by Sam et al.

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[16]. Chen et al. [17] observed such activity with site selectivity by a V^{V}-complex with the Schiff base of 2-hydroxy-1-naphthaldehyde and L-phenylalanine, and Kwong et al. [18] studied another example of sequence-specific photocleavage by a V^{V}-peroxo complex. Recently, Sasmal et al. [19] also reported three new V^{IV}-complexes with DNA photocleavage activity.

In all the mentioned cases, the DNA cleavage activity is observed only in the presence of an activating agent, usually an oxidizing agent (oxone, hydrogen peroxide), or is induced by photo irradiation, the only exceptions being complexes including phen as a ligand. In a previous study [20] when comparing the nuclease activity of several different types of V^{IV}-complexes we found $V^{V}O(acac)_2$ and $V^{IV}O(phen)_2$ to be the most active in DNA cleavage.

$V^{IV}O(acac)_2$ was first prepared in 1876 [21] and since then it has been known as a precursor for the synthesis of V complexes [22] and as a catalyst for epoxidations, namely selective epoxidation of allylic alcohols [23–25]. $V^{IV}O(acac)_2$ was found to have insulin-enhancing activity, with apparent low toxicity [9,26], and it is thus considered as a potential therapeutic agent for diabetes mellitus [27]. Its potential has motivated recent studies regarding its electrochemical properties and solution structure [9,28–30]. Our interest is centered on its behaviour towards DNA, both as a promising drug candidate for the treatment of diabetes mellitus and as an inorganic nuclease.

In all studies reported in the literature so far the nature of the active vanadium species in DNA cleavage is not clearly understood. Nuclease activity studies are carried out at micromolar metal ion concentrations, at which the metal ion speciation is not easily accessible. In this work we compare the nuclease activity of four V^{IV}-complexes with similar β-diketonate ligands: $V^{IV}O(acac)_2$ **1**, $V^{V}O(hd)_2$ **2**, $V^{IV}O(acac\text{-}NH_2)_2$ **3,** and $V^{IV}O(acac\text{-}NMe_2)_2$ **4** (Fig. 1). Our aim is to evaluate the nuclease activity of these V complexes, and to try to establish which is/are the active species in DNA cleavage by these compounds.

2. Experimental

2.1. Synthesis

$V^{IV}O(acac)_2$ **1** was obtained from ACROS, and was used without further purification. Complexes **2**–**4** were prepared according to literature procedures [31,32].

Complex **2**: 78.2% yield; elemental analysis (%): calculated for $[VO(C_7H_{11}O_2)_2]$ (experimental): C = 52.34 (52.2); H = 6.90 (7.2); IR(KBr pellet): ν(V=O)/cm^{-1} = 998 (expected 998 [31]). Complex **3**: 74.3% yield; elemental analysis (%): calculated for $C_8H_{12}N_2O_5V$ (experimental): C = 35.97 (35.8); H = 4.53 (4.6); N = 10.49 (10.3) IR(KBr pellet): ν(V=O)/cm^{-1} = 979 (expected 981 [32]). Complex **4**: 72.1% yield; elemental analysis (%): calculated for $C_{12}H_{20}N_2O_5V$ (experimental): C = 44.59 (44.6); H = 6.24 (6.5); N = 8.67 (9.1) IR(KBr pellet): ν(V=O)/cm^{-1} = 987 (expected 988 [32]). Solutions of monovanadate and decavanadate were prepared as described in the literature [33,34].

2.2. DNA cleavage activity

The plasmid DNA used for gel electrophoresis experiments was pA1, which consists of a full-length cDNA from Cytochrome P450 CYP3A1 inserted in the PBS plasmid vector (pBluescribe, Stratagene, UK) and described elsewhere [35]. Plasmid DNA was amplified in *Escherichia coli* DH5α and purified using PureYield™ Plasmid Midiprep System from Promega. Linear DNA was obtained by digestion of pA1 with HindIII and used as a reference in agarose gel electrophoresis.

DNA concentration per nucleotide base pair (bp) was determined by UV absorption at 260 nm using the extinction coefficient of 13,200 M^{-1} cm^{-1} bp^{-1}.

Typically, a 100 μM mother solution of vanadium complex in deionized MilliQ® water was freshly prepared for each experiment.

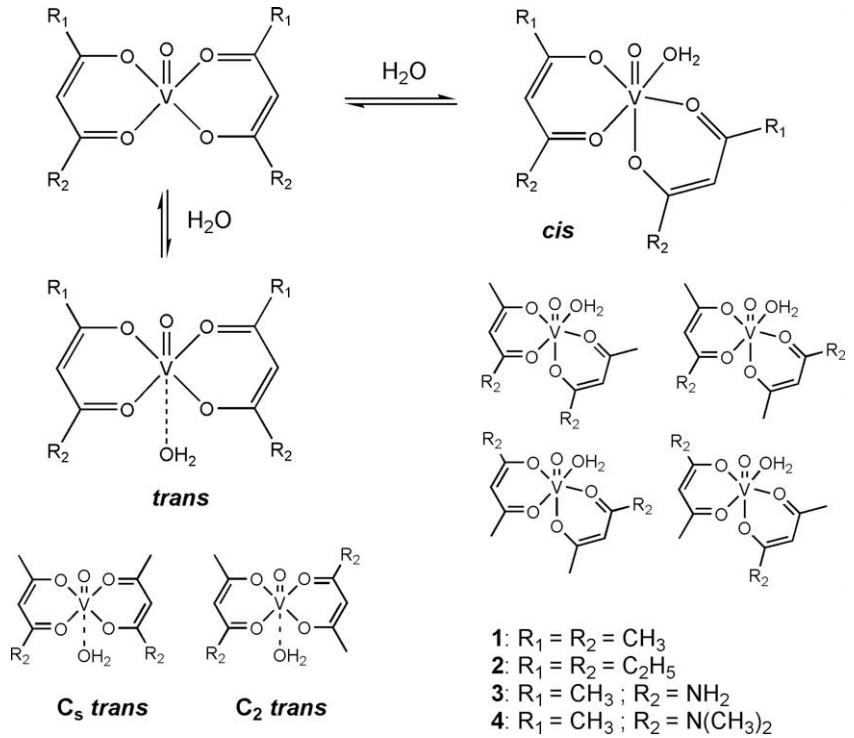

Fig. 1. Structures and possible isomers of complexes **1**–**4**. Coordination of a water molecule either in *trans* or *cis* position relative to the oxo group leads to two theoretically possible isomers of **1** and **2**. For **3** and **4**, two *trans* and four *cis* isomers are theoretically possible. Crans et al. [32] report two ν(V=O) bands for solid complexes **3** and **4**, which should correspond to the two trans isomers of symmetry C_s and C_2. We also observed similar bands in the IR spectra of these compounds, but in our opinion the bands cannot be undoubtly assigned to ν(V=O) absorptions.