



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

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### ABSTRACT

The DNA cleavage activity of several  $\beta$ -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),  $V^{IV}O(acac-NH_2)_2$  (**3**, Hacac-NH<sub>2</sub> = acetoacetamide) and  $V^{IV}O(acac-NMe_2)_2$  (**4**, Hacac-NMe<sub>2</sub> = N,N-dimethylacetamide) 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**  $\gg$  **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.

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### 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 therapeutic 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  $V^{IV}$ -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  $V^{IV}$ -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  $V^{IV}VO(acac)_2$  and  $V^{IV}VO(phen)_2$  to be the most active in DNA cleavage.

$V^{IV}VO(acac)_2$  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].  $V^{IV}VO(acac)_2$  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  $\beta$ -diketonate ligands:  $V^{IV}VO(acac)_2$  **1**,  $V^{IV}VO(hd)_2$  **2**,  $V^{IV}VO(acac-NH_2)_2$  **3**, and  $V^{IV}VO(acac-NMe_2)_2$  **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

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

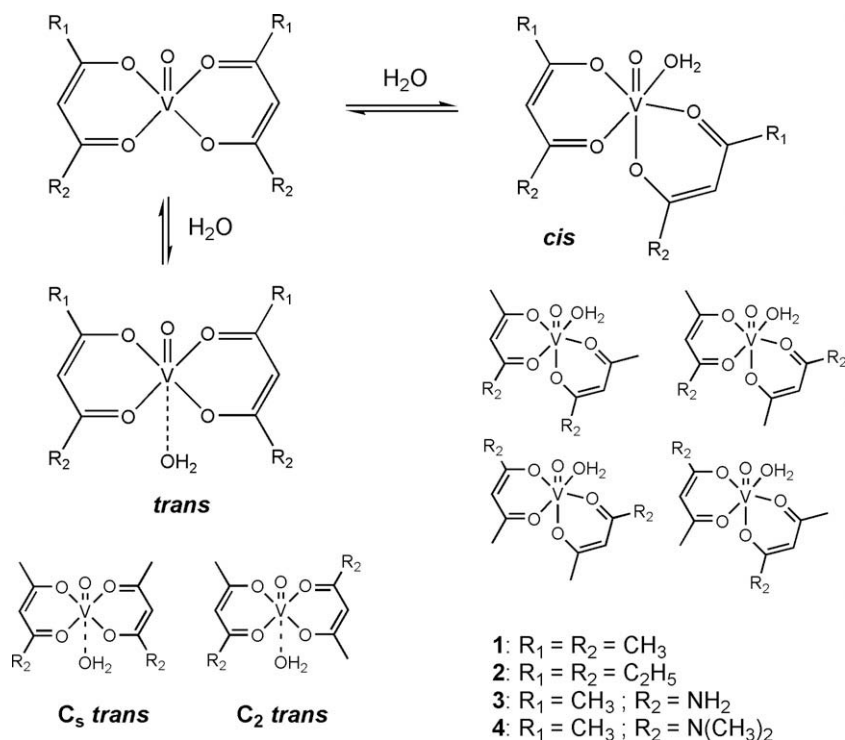
Complex **2**: 78.2% yield; elemental analysis (%): calculated for  $[VO(C_7H_{11}O_2)_2]$  (experimental): C = 52.34 (52.2); H = 6.90 (7.2); IR(KBr pellet):  $\nu(V=O)/cm^{-1}$  = 998 (expected 998 [31]). Complex **3**: 74.3% yield; elemental analysis (%): calculated for  $C_8H_{12}N_2O_5V$  (experimental): C = 35.97 (35.8); H = 4.53 (4.6); N = 10.49 (10.3) IR(KBr pellet):  $\nu(V=O)/cm^{-1}$  = 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):  $\nu(V=O)/cm^{-1}$  = 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* DH5 $\alpha$  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  $\mu M$  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  $\nu(V=O)$  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 undoubtedly assigned to  $\nu(V=O)$  absorptions.

When necessary, dissolution was completed using sonication. For experiments varying complex concentration this solution was diluted immediately before addition to the sample/reaction mixture.

DNA cleavage activity was evaluated by monitoring the conversion of supercoiled plasmid DNA (Sc) to nicked circular DNA (Nck) and linear DNA (Lin). Each reaction mixture was prepared by adding (in this order) 6  $\mu\text{L}$  of water, 2  $\mu\text{L}$  (0.2  $\mu\text{g}$ ) of supercoiled pA1 DNA, 2  $\mu\text{L}$  of 100 mM stock pH7 buffer solution and 10  $\mu\text{L}$  of the aqueous solution of the complex. The pH buffer was  $\text{Na}_2\text{HPO}_4/\text{HCl}$ , unless stated otherwise. Tris/HCl, hepes/HCl or mops/HCl were also used. The final reaction volume was 20  $\mu\text{L}$  and the final metal concentration varied from 0.3 to 100  $\mu\text{M}$ . The final buffer concentration was 10 mM. When the reaction involved additional agents (such as radical scavengers, activating agents or excess of ligand), the initial volume of water was reduced to 4  $\mu\text{L}$ , and 2  $\mu\text{L}$  of a solution of the agent were added before the metal complex; the final concentration was 40 mM for the radical scavengers, and 200  $\mu\text{M}$  for oxone and MPA. For additions of excess ligand, a 64 mM stock solution of the ligand in MilliQ<sup>®</sup> water was prepared and diluted as needed. Stock solutions of  $\sim 1$  mM hydrogen peroxide were freshly prepared from 30%  $\text{H}_2\text{O}_2$  (Panreac), their concentration measured spectrophotometrically ( $\epsilon_{230\text{ nm}} = 74\text{ M}^{-1}\text{ cm}^{-1}$  [36]), and diluted as needed immediately before addition to the mixture.

Samples were typically incubated for 1 h at 37  $^\circ\text{C}$ , wrapped up in aluminium foil. After incubation, 5  $\mu\text{L}$  of DNA loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in water) were added to each tube and the solution was loaded onto a 1% agarose gel in TBE buffer (89 mM Tris–borate, 1 mM EDTA, pH 8.3) containing ethidium bromide (1  $\mu\text{g}/\text{mL}$ ). Controls of non-incubated and of linearized plasmid were included in both extremes of an 18-well gel plate. The electrophoresis was carried out for 2 h at 120 V. Bands were visualised under UV light and photographed using an AlphaImager (Alpha Innotech). The photos chosen for this publication were rearranged to show only the relevant samples. All samples in each figure were obtained from the same run. Peak areas were measured by densitometry using AlphaEaseFC<sup>TM</sup> Software from Alpha Innotech. Peak areas for the Sc form were corrected using the factor 1.47 to account for its lower staining capacity by ethidium bromide [37] and used to calculate the percentage (%) of each form (Sc, Nck and Lin). Standard deviation for repeatability ( $s_r$ )<sup>1</sup> for the peak area within the same gel was estimated as 0.8% for the Sc form and 6% for the Nck form. The  $s_r$  of the whole experimental procedure, including the preparation of solutions and incubation was 10%, 6% and 3%, for the Sc, Nck and Lin forms, respectively.

Experiments under inert atmosphere were carried out using Schlenk techniques. Solvents for the preparation of vanadium complex solutions were degassed prior to the dissolution of the complex under nitrogen. Eppendorfs containing the DNA and buffer solutions were submitted to vacuum and left under  $\text{N}_2$ -atmosphere before the addition of the complex solution. Eppendorfs were incubated inside a Schlenk under nitrogen, at 37  $^\circ\text{C}$ , for 1 h.

Experiments under UV light were carried out preparing the reaction mixtures as described, and incubating them in open Eppendorfs under a 8 W blacklight blue 370 nm lamp for 1 h. Experiments in the dark were carried out preparing all solutions in a dark room with red light, and incubating the reaction mixtures for 1 h at 37  $^\circ\text{C}$  wrapped in aluminium paper.

<sup>1</sup>  $s_r$  measures the random variability within each experiment.  $s_r$  values were estimated from analysis of variance (single factor ANOVA) of duplicates from 11 different runs.  $s_r$  for the peak area was estimated from duplicates of plasmid DNA (not incubated).  $s_r$  for the whole procedure was estimated from duplicates of samples incubated with metal complex.

### 2.3. Circular dichroism (CD) and UV-visible (UV-vis) absorbance spectra

CD spectra were recorded on a Jasco J-720 spectropolarimeter with a 180–800 nm photomultiplier using 1 cm path-length cylindrical quartz cells. Samples of appropriate concentration of pA1 DNA and **1** were prepared in phosphate-buffered saline (PBS). Spectra were recorded at  $t = (25 \pm 1)^\circ\text{C}$  in the 200–400 nm range. Stock (200  $\mu\text{M}$ ) solutions of **1** in 100% PBS were always prepared immediately before use. PBS was purchased from Sigma (PBS is a pH 7.4 phosphate buffered medium with controlled ionic strength: 10 mM phosphate buffer, 2.7 mM KCl, 137 mM NaCl). After running each CD spectrum, a small volume of sample was stored at  $-20^\circ\text{C}$  for analysis by agarose gel electrophoresis.

UV-vis spectra were recorded at room temperature on a Hitachi U-2000 double beam spectrometer with 1 or 4 cm path-length quartz cell in the 380–900 nm range. Complex stock solutions of appropriate concentrations in water:DMSO media (up to 20% DMSO depending upon solubility) were freshly prepared immediately before use and diluted with pH 7 buffer (PBS or hepes medium) to obtain 2 mM working solutions with a max. content of 10% DMSO overall. Hepses medium refers to a buffered medium with controlled ionic strength: 10 mM hepes, 4 mM KCl, 132 mM NaCl adjusted to pH 7. Deionized water was always used in these preparations. All reagents used were p.a. grade; DMSO was purchased from Fisher, KCl and NaCl from Merck.

### 2.4. Spin-trapping

EPR spectra were recorded at room temperature on a flat cell with a Bruker ESR-ER 200D X-band spectrometer. DMPO from Sigma was used as the spin trap agent for radicals.

DMPO (20 mM) was added to a 1.5 mM solution of **1** in PBS buffer pH 7.4 and 1% DMSO, and the sample was stirred under air and kept at room temperature. The EPR spectra were recorded: (i) immediately after preparation, (ii) after 1 h and (iii) after 18 h.

### 2.5. Atomic force microscopy (AFM)

Samples for AFM were prepared and incubated similarly to samples for gel electrophoresis, with the following differences: plasmid DNA pBR322 from Roche was used, and the total volume for each sample was 50  $\mu\text{L}$ . Following the digestion, each sample was evaporated over a mica surface, after the addition of  $\text{MgCl}_2$  to facilitate the adhesion of DNA to the surface. Images were obtained with a NANOSCOPE III MULTIMODE AFM, of Digital Instruments Inc. operating in tapping mode.

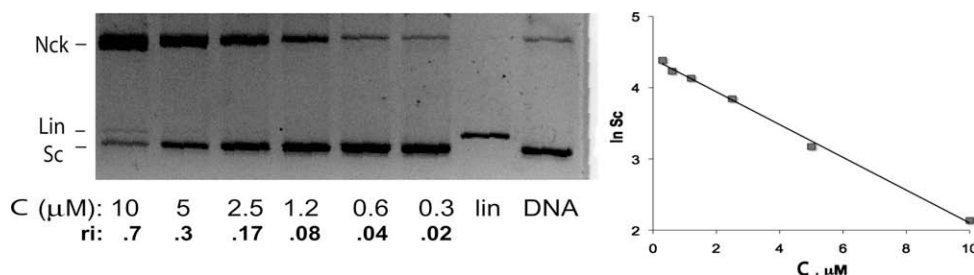
### 2.6. Fluorescence spectroscopy

Fluorescence spectra were recorded on a Jasco FP-777 spectrofluorometer using a 1 cm cell. The emission was scanned from 350 to 470 nm at an excitation wavelength of 305 nm, and variable excitation was scanned from 250 to 350 nm at 407 nm emission. Fifty milliliters of 10 mM benzoic acid (Riedel, 99.5%) were added to 50 mL of 200  $\mu\text{M}$  of **1**, in 10 mM pH 7 phosphate buffer. The solution was stirred under air at room temperature for 3 h. Samples were taken every 10 min and their emission and excitation spectra measured.

## 3. Results and discussion

### 3.1. DNA cleavage by $\text{V}^{\text{VO}}(\text{acac})_2$

DNA degradation by **1** is detectable as single-strand cleavage for metal concentration as low as 1.2  $\mu\text{M}$ , which corresponds to a



**Fig. 2.** Effect of concentration of **1** on DNA cleavage. *Left*: agarose gel electrophoresis evolution of plasmid pA1 degradation after incubation with **1**. “Lin” and “DNA” are the controls for linearized and for undigested plasmid. *Right*: the same results, measured as logarithm of the percentage of the Sc form. From the least squares linear regression fit to this data,  $\ln Sc = (4.40 \pm 0.09) - (0.23 \pm 0.02) C$  for a 95% confidence level.  $C$  refers to the complex concentration, in  $\mu\text{M}$ .

$ri = 0.08$  (Fig. 2)  $\{ri = [\text{metal}]:[\text{DNA (bp)}]\}$ . It is clear from Fig. 2 that as the concentration of **1** increases there is a higher extent of DNA nicking, shown by the decrease in the intensity of the Sc band and the increase in the Nck band. At ca.  $10 \mu\text{M}$  ( $ri = 0.7$ ) the band of linear DNA is visible, indicating some double-strand cleavage. For higher concentrations (Fig. 5B – 1), the amount of the Sc form decreases, as well as that of the Nck form, and the amount of the linear DNA increases.

To our knowledge this is the first reported example of such an efficient DNA cleavage by a  $V^{IV}$ -complex not containing phen or other specific DNA-binder as ligand. However, a comparison of the efficiency of DNA cleavage by the different nucleases reported in the literature is difficult due to the large variety of reaction conditions used.

A measure of the efficiency of artificial nucleases may be given by  $S/M$ , where  $S$  is the average number of strand breaks made in each plasmid per hour at  $37^\circ\text{C}$ , and  $M$  is the metal micromolar concentration [38].  $S$  can be calculated as  $-lnf$ , where  $f$  is the fraction of supercoiled plasmid [39], when only the Sc and Nck forms are observed.<sup>2</sup>

For complex **1**  $S/M$  is given by the slope of the curve in Fig. 2,  $0.23 \mu\text{M}^{-1}$ . This value is similar to that of Cu-neamine [38], one of the most efficient inorganic DNA hydrolytic cleavers reported [40].

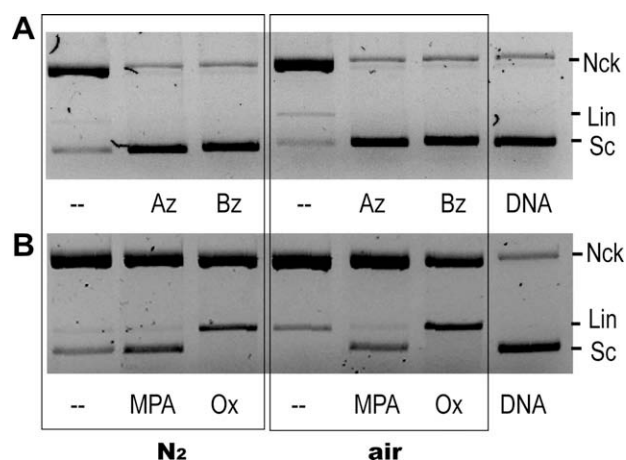
### 3.2. Studies on the mechanism of action of $V^{IV}O(\text{acac})_2$

#### 3.2.1. Effect of light, air and activating agents

Experiments carried out in the dark gave results similar to those done either under natural light or incubated under a 370 nm lamp. This indicates that photocleavage is not an important process in DNA degradation by  $V^{IV}O(\text{acac})_2$ .

Under inert atmosphere the activity of **1** decreased slightly, nevertheless exhibiting a significant effect (Fig. 3). These results indicate that air does not have a primary role in the nuclease activity of **1** which is possibly taking place through more than one mechanism in parallel, one at least involving atmospheric oxygen.

The effect of the presence of either an oxidizing or a reducing agent was tested by adding oxone or MPA (Fig. 3B). Oxone enhances the complex nuclease efficiency, and it is observed that supercoiled DNA is totally broken into its Nck and linear forms. The activating effect of oxone is similar both under air and inert atmosphere. In contrast the reducing agent MPA inhibits the nuclease activity, although some degradation of the Sc form into the Nck form still takes place. This inhibiting effect also takes place to the same extent under air and under inert atmosphere, but it appears to be less dramatic under  $N_2$  because activity is already low in the absence of any agent.



**Fig. 3.** Effect of air on the nuclease activity of **1**. Experiment A was carried out with  $ri = 0.8$  and additions of radical scavengers sodium azide (Az) and sodium benzoate (Bz). Experiment B was carried out with  $ri = 1.7$  and with additions of mercaptopyropionic acid (MPA) and Oxone (Ox). Samples on the left box were incubated under nitrogen atmosphere; samples on the right box were incubated under air. The first sample in each set (–) was incubated with **1** in the absence of any agent. The last lane to the right (DNA) is the control for undigested plasmid.

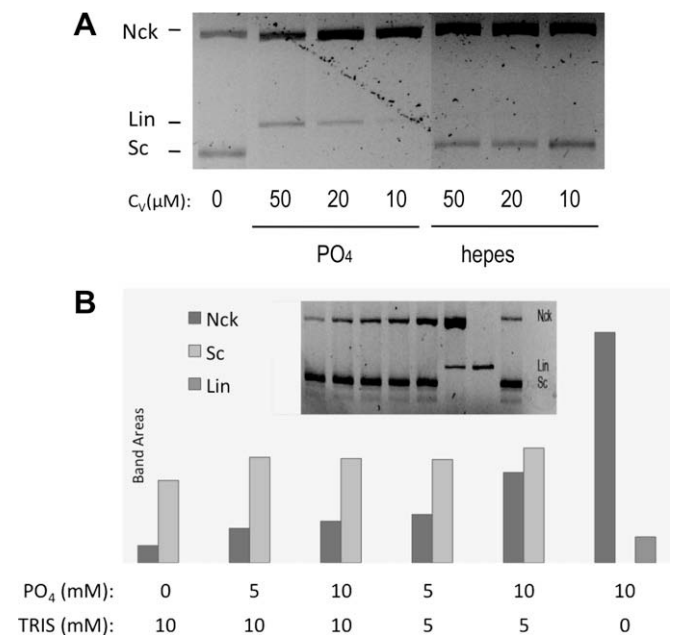
#### 3.2.2. Effect of radical scavengers and spin-trapping

As a first approach to detect the involvement of ROS, several known OH radical scavengers [41] were added to the reaction mixture: D-mannitol, ethanol, DMSO and sodium benzoate. The addition of *t*-butanol [42] and sodium azide [38] was also tested. Results for sodium benzoate and sodium azide are presented in Fig. 3A. DNA cleavage is completely inhibited in all cases, and such an inhibition suggests a mechanism involving the formation of radicals.

To confirm this hypothesis, spin-trapping experiments with DMPO (5,5-dimethylpyrrolidine-N-oxide) were carried out. DMPO reacts with several radicals to form adducts with different EPR spectra. Its reaction with the OH radical produces DMPO–OH and a characteristic four line EPR spectrum [41]. EPR spectra of millimolar solutions of **1** with DMPO showed the 8-line signal typical of  $V^{IV}$ -complexes ( $I = 7/2$ ,  $S = 1/2$ ). The sample was stirred overnight under air to promote the oxidation of  $V^{IV}$ , but after the disappearance of the  $V^{IV}$ -signal still no resonances for the DMPO–OH adduct were detected. These results do not exclude the formation of OH or other radicals. The detection limit for OH radicals by spin trapping with DMPO would be  $\sim 10^{-6} \text{ M}$ , but the presence of a residual signal from  $V^{IV}$  makes the detection of other signals difficult and the detection limit higher. It is also expected that OH radicals would react with DMSO to yield methyl radicals. These react with  $O_2$  faster than with DMPO, so dissolved oxygen would compete with DMPO for the methyl radicals produced [41]. It is possi-

<sup>2</sup> A Poisson distribution is used in the calculation of  $S$ , assuming the nicks are randomly distributed among the DNA population.





**Fig. 4.** Effect of the nature of the pH buffer on DNA cleavage by **1**. (A) Incubation under phosphate (PO<sub>4</sub>) or hepes buffers and (B) DNA cleavage efficiency of **1** (50 μM) after incubation in solutions buffered with mixtures of phosphate and tris buffers. The inset contains the corresponding agarose gel, which also shows the controls for linear and native DNA on the two rightmost positions.

ble that, if there is a slow generation of radicals in solutions of **1**, it would not be detected by this method.

A distinct method was also used. Hydroxylated aromatic compounds are often used to detect OH radicals [41] and may be analyzed by fluorimetry, a highly sensitive technique. The reaction of benzoate with OH radicals produces *o*- and *p*-hydroxybenzoates with a strong fluorescent emission at 407 nm when irradiated at 305 nm [43]. When 100 μM of **1** were added to 250 μM of sodium benzoate in 10 mM pH 7 phosphate buffer, the intensity of fluorescence increased gradually with time, and the wavelength of maximum emission shifted slightly from 410 nm to lower wavelengths (Fig. S1 in the Supplementary material). These results suggest the formation of OH radicals in V<sup>VO</sup>(*acac*)<sub>2</sub> solutions in pH 7 phosphate medium, and support the findings by electrophoresis.

### 3.2.3. pH buffer and solvation effects

The nature of the pH buffer was found to be determinant for the nuclease activity of **1**. Experiments made in solutions buffered with tris or with the “Good” buffers (hepes, mes or mops) showed a much less efficient DNA cleavage than experiments carried out in a phosphate buffered medium. As an example, Fig. 4A compares the efficiency of **1** under phosphate and hepes buffers. The experiment in Fig. 4B was done buffering the reaction medium with mixtures of phosphate and tris. The reaction is always inhibited in the presence of tris, even when phosphate is in a two-fold excess, but proceeds with a strong linearization of DNA when tris is absent. We emphasize that these experiments do not show an activating effect of phosphate, but instead they show an inhibiting effect of tris.<sup>3</sup>

<sup>3</sup> Incubating plasmid DNA with **1** in the absence of any buffer shows extensive DNA degradation (Fig. S2 in the Supplementary material). Not controlling the pH makes the interpretation of such an experiment difficult. Most likely the pH will change with time during the reaction, and this may affect cleavage efficiency. Nevertheless, results show complete degradation of Sc DNA by **1** in the absence of phosphate, suggesting that phosphate does not play a central role in the nuclease activity of these complexes.

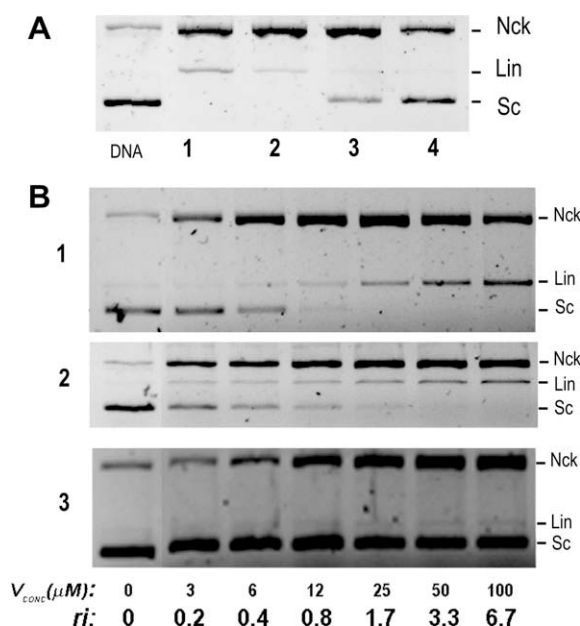
Amine based buffers are protonated at pH 7, and thus positively charged. An increase in the concentration of positive ions in solution stabilizes DNA by an electrostatic effect. Positive ions counterbalance the negative charges in the phosphate backbone, and thus reduce the repulsion between the two strands of the DNA double chain. Buffers such as tris, mops and hepes are known to form adducts with DNA, shielding it from the action of nucleases [44]. If the inhibiting effect is due to a purely electrostatic stabilization, it should be observed when the concentration of an electrolyte such as NaCl is increased. Moreover, experiments carried out in 10 mM phosphate buffer containing 10 mM or 137 mM NaCl showed no differences when compared to experiments with no added NaCl, indicating that the inhibiting effect of the organic buffers cannot be purely electrostatic. If this effect contributes at all to the inhibition of DNA cleavage, it is not the determining factor.

Unlike phosphate, tris and hepes buffers are weak OH radical scavengers [41,45]. When present in a DNA solution at pH 7, the positively charged buffer molecules will lay near the negatively charged DNA phosphate backbone. The concentration of buffer molecules will be higher near the DNA double chain than in the bulk solution, so its scavenging effect will be much stronger. We therefore expect that organic buffers will have a shielding effect on DNA, protecting it from free radicals.

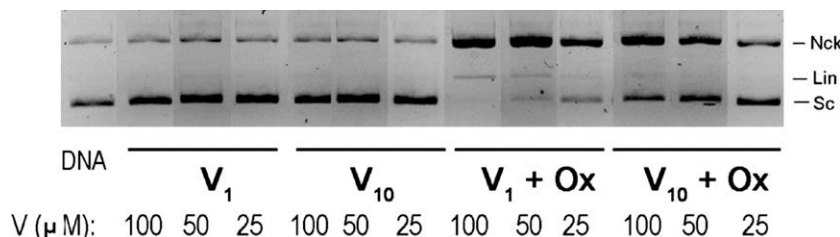
### 3.3. DNA cleavage by V<sup>VO</sup>(*acac*)<sub>2</sub> related derivatives

Fig. 5A compares results of DNA cleavage by complexes **1**, **2**, **3** and **4** at a fixed *ri* of 1.7 under the same incubation conditions. All complexes are active. Complexes **1** and **2** exhibit a similar activity, much higher than that of **3** and **4**. In fact, **1** and **2** completely degrade Sc DNA into the Nck and Lin forms, while **3** and **4** do it only partially, and were not able to linearize the DNA. In the same gel, the Lin form is present in slightly higher amount in the lane associated with **1**, indicating a stronger double-strand cleavage ability for complex **1**. It is thus apparent that **1** is a more efficient nuclease agent than **2**, while complex **4** is the least efficient of all tested.

Fig. 5B compares the activity of complexes **1**–**3** at several *ri* (from 0.2 to 6.7). Complete decomposition of Sc DNA into its Nck



**Fig. 5.** (A) Comparison of DNA cleavage after incubation with complexes **1**–**4** at the same value of *ri* (1.7) and (B) effect of the complex concentration on DNA cleavage, for complexes **1**–**3**.



**Fig. 6.** Effect of monovanadate ( $V_1$ ) and decavanadate ( $V_{10}$ ) on pA1 DNA at the specified concentrations (25, 50 and 100  $\mu\text{M}$ , measured for V atoms,  $r_i$  1.7, 3.3 and 6.7, respectively) and after the addition of oxone (Ox).

and Lin forms is achieved at the lowest  $r_i$  (1.7) by complex **1**, while with complex **2** the same is only observed for  $r_i = 3.3$ . Complex **3** clearly promotes single-strand cleavage at  $r_i$  6.7, but it still leaves a significant amount of Sc form, confirming its lower efficiency in DNA cleavage.

### 3.4. DNA cleavage by monovanadate and decavanadate

$V^{IV}$ -complexes in solution can be involved, particularly at low concentrations and neutral pH, in oxidation and/or hydrolytic processes yielding as final products free vanadate ( $V^V$ ) and the unbound ligand. One important aspect to understand is if the products of the oxidation or hydrolysis of **1** are the active species in DNA degradation. To answer this question, the most usual product of oxidation of dilute aqueous solutions of  $V^{IV}$ -complexes, monovanadate, was tested (Fig. 6). The results show that monovanadate by itself does not affect DNA. Although it can cleave DNA in the presence of oxone, it is much less efficient than **1**. Therefore, we can rule out monovanadate as the species responsible for the nuclease activity of **1**.

Decavanadate is a kinetically stable species which may form when vanadium concentration is relatively high in a slightly acidic medium. When pH and V concentration are changed from the thermodynamically favourable ones it decomposes with a very slow kinetics [34,46]. Like the monomeric species, decavanadate ( $V_{10}$ ) does not show any activity in the absence of an activating agent (Fig. 6). In the presence of oxone, it does show some activity, but much lower than that of monovanadate.  $V_{10}$  clusters are bulky and compact, and it is likely that the activity observed is due to some monovanadate formed by the decomposition of  $V_{10}$  at pH 7, which is much faster at 37 °C than at room temperature [47], and not due to the decavanadate itself.

The nuclease activity measured for solutions of complexes **1–4** left under air for 24 h was always lower than that of fresh solutions. This observation also supports the assumption that decomposition products are not the species responsible for DNA cleavage.

### 3.5. Atomic force microscopy

Fig. 7 shows atomic force microscopy images of plasmid DNA after incubation with complexes **1** and **2**, at  $r_i$  0.6 and 1.3, in phosphate and hepes buffers. According to the gel electrophoresis shown in Fig. 5B, after incubation in phosphate buffer at these  $r_i$  values, the complete degradation of Sc DNA into the linear and nicked forms is expected. Nevertheless, no significant degradation of Sc is seen in the AFM images in Fig. 7. The reason for this is that the plasmid used for AFM experiments was commercial pBR322 stored in 10 mM tris and 1 mM EDTA. This was diluted 1:10 in the reaction mixture, so the reaction took place not only in 10 mM phosphate buffer, but in a medium that contained also 1 mM tris. Tris buffer was found to quench DNA cleavage by these complexes (see above). EDTA (which has a strong ability to bind  $V^{IV}$ ) is also present at 100  $\mu\text{M}$  concentration, so it is plausible that

an important fraction of the total vanadium is complexed by EDTA, only a small fraction remaining as  $V^{IV}O(\text{acac})_2$ .<sup>4</sup>

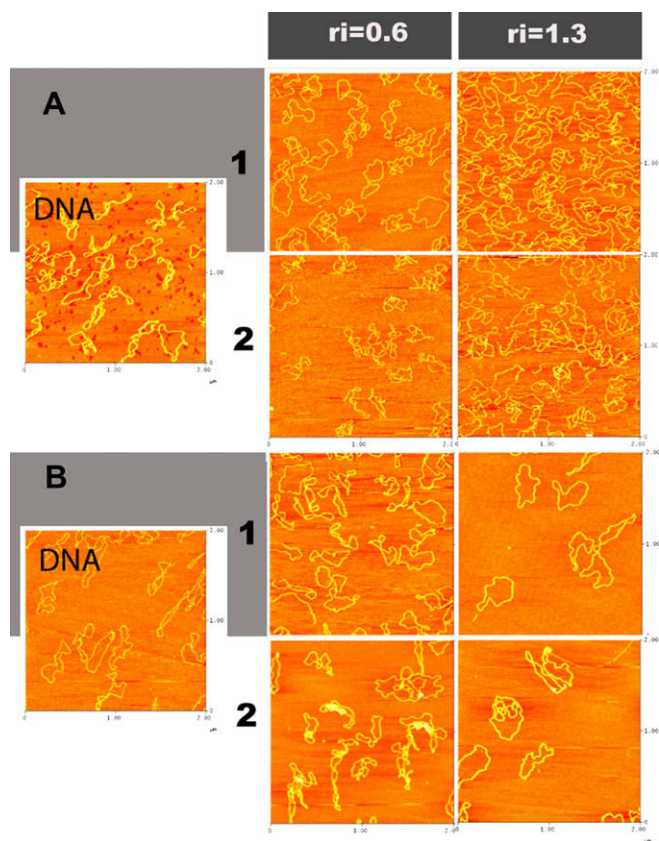
Although, for the reasons mentioned above, the AFM images in Fig. 7 cannot be directly compared with the electrophoresis results, they are included here to emphasize that there is a solvation effect of the buffer solution on DNA, which is dependent upon complex concentration. The presence of complex seems to concentrate the DNA molecules in phosphate buffer (Fig. 7A), while in hepes buffer it makes them more widely dispersed (Fig. 7B). This is observed for both complex **1** and **2**, and the effect increases with complex concentration. Some aggregation of DNA molecules seems to take place in DNA incubated in hepes with **2**, but not with **1**.

### 3.6. Effect of the stability of the complex

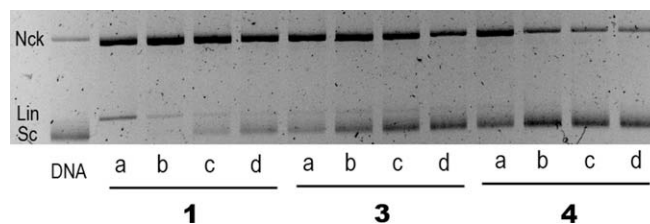
The stability of complexes **1–4** in aqueous solution was evaluated by visible absorption spectroscopy, and Fig. 8 shows the changes observed with time. Complex **1** is reasonably stable for at least 24 h in aqueous PBS solution; some modifications are detected after 2 h, possibly mainly due to  $V^{IV}O$ -oxidation. Complex **2** is also stable, although after ca. 4 h measurable changes (hydrolysis/oxidation) are already observed. Complexes **3** and **4** hydrolyse/decompose very significantly in less than one hour. This is in agreement with previous studies on the stability of these complexes [9,32] which follows the order **1–2**  $\gg$  **3–4**.

Speciation studies [32] show that when ligand is added in a 80-fold excess at pH 6, **3** and **4** are over 90% in the  $V^{IV}OL_2$  form. According to this, incubations were carried out in solutions containing complexes **3** and **4** where the complex was stabilized by addition of an excess of ligand up to  $\sim 300$  (Fig. 9). Experiments with **1** were performed in parallel as a control. No increase of nuclease activity was observed. Instead, there is a clear decrease of the nuclease activity when acac is added to **1**, and a slighter one when excess ligands are added to **3** and **4**. This decrease might suggest that another species other than  $V^{IV}OL_2$  is the active one, but this is unlikely. The activity continues to decrease at a similar rate when the L/M increases to 164 and 324, when  $V^{IV}OL_2$  is already by far the predominant species. This result is better explained if the mechanism is radical and at high concentrations the excess ligand acts as a radical scavenger.

<sup>4</sup> Being an excellent chelating agent, EDTA will surely affect the speciation of vanadium in solution, removing the coordinated acac ligands to a considerable extent. However, although the stability constant for  $V^{IV}O(\text{EDTA})$  ( $\log \beta = 18.8$ ) is higher than for  $V^{IV}O(\text{acac})_2$  ( $\log \beta_2 = 15.6$ ) [J. Selbin, Chem. Rev. 65 (1965) 153–175], the molar fraction of deprotonated acac at pH 7 is much higher (2%) than that of  $\text{EDTA}^{4-}$  (0.05%), and thus the conditional constant corrected for pH 7 is similar for both complexes ( $\log \beta' [\text{VO}(\text{acac})_2] = 15.8$  and  $\log \beta' [\text{VO}(\text{EDTA})] = 15.5$ ). Both ligands are at similar analytical concentrations in the reaction solution, so a mixture of both complexes is expected to be present.



**Fig. 7.** AFM images of pBR322 plasmid DNA after incubation with complexes **1** and **2** in phosphate buffer (A) and hepes buffer (B), at metal concentrations of 10 and 20  $\mu\text{M}$  ( $ri = 0.6$  and  $1.3$ ).



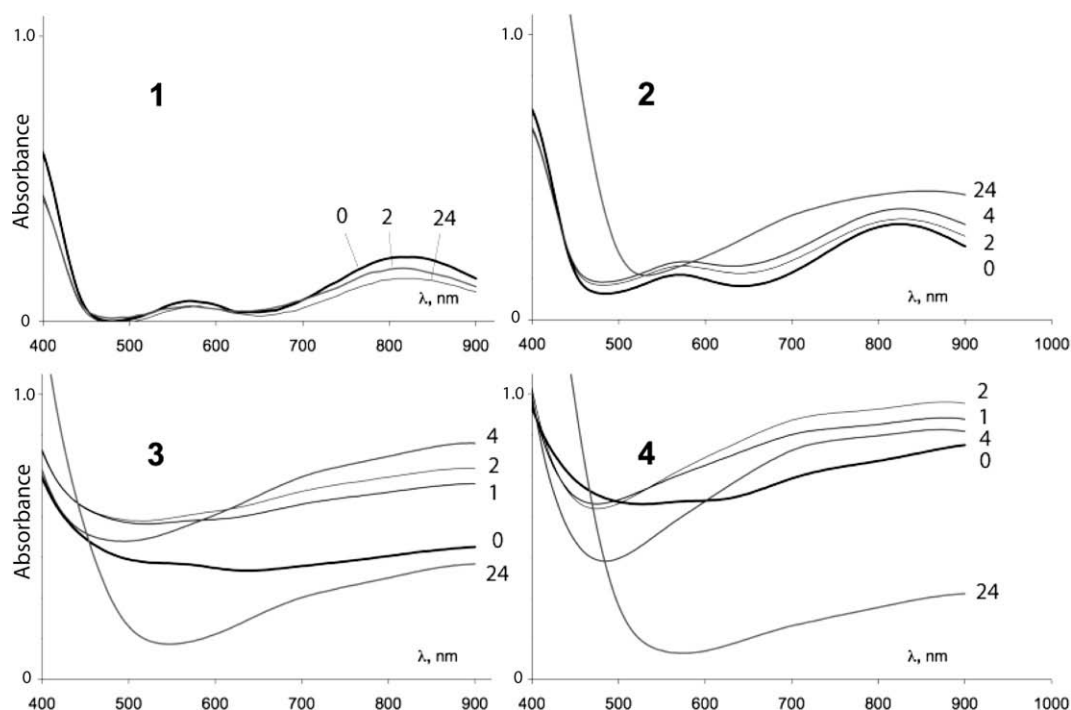
**Fig. 9.** Effect of excess ligand in the nuclease activity of complexes **1**, **3** and **4**, for ligand-to-metal (L/M) ratios of (a) **2** (the  $\text{V}^{\text{IV}}\text{OL}_2$  complex) (b) **82** (c) **164** and (d) **324**. The total vanadium concentration is 50  $\mu\text{M}$ .

### 3.7. Circular dichroism measurements

The circular dichroism spectrum of pA1 DNA in PBS buffer shows a negative band with  $\lambda_{\text{max}} = 246$  nm and a positive band at  $\lambda_{\text{max}} = 275$  nm (Fig. 10), characteristics of right-handed B-form DNA [48,49]. Addition of **1** (100  $\mu\text{M}$ ,  $ri = 0.7$ ) induces no significant changes in the CD spectra after 2.5 h of incubation under air at room temperature. After 19 h of incubation the intensity of the negative band increases and that of the positive band decreases, both by ca. 10%. The changes observed in the pA1 DNA bands are consistent with its degradation. These results in the 220–230 nm range therefore indicate some degradation of the DNA molecule without extensive change of either helicity or base-stacking.

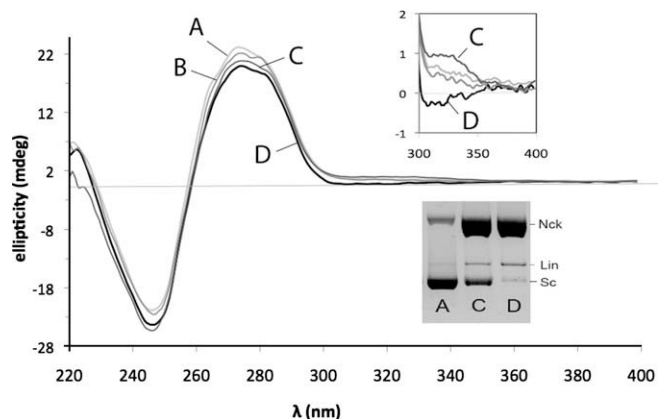
Agarose gel electrophoresis of these samples showed significant degradation of DNA after incubation with **1**: the Sc form was degraded by 77% after room temperature incubation for 19 h at  $ri$  0.7, and by 98% after increasing  $ri$  to 1.8 with fresh complex, and incubating further 100 min at 37  $^\circ\text{C}$ .

The observation of a CD spectrum in the 450–900 nm of solutions containing DNA and  $\text{V}^{\text{IV}}\text{O}(\text{acac})_2$  would clearly confirm a close contact between vanadium and DNA, but no induced CD signal on the



**Fig. 8.** Stability of complexes **1–4** in aqueous solution at pH 7: change with time of the UV-vis absorbance spectra of 2 mM solutions of complexes in (1) PBS (1% DMSO), and (2–4) hepes (10% DMSO). Numbers indicate time (h) after dissolution.





**Fig. 10.** Circular dichroism spectra of (A) pA1 in PBS, (B) pA1 + **1** in PBS,  $r_i = 0.7$ , after 2.5 h of incubation at room temperature, (C) the same solution after incubation at room temperature for 19 h; (D) the same solution after addition of **1** ( $r_i = 1.8$ ) and incubation at 37 °C for 100 min (the intensity was corrected for a dilution factor of 1.8 after the addition of **1**). The insets show an enlargement of the spectra of A, B, C and D and the results of agarose gel electrophoresis of samples A, C and D.

d–d transitions of **1** was detected even when using 5 cm path-length cells either with pA1 plasmid DNA or calf thymus DNA. This indicates that at pH ~ 7 no coordination to vanadium occurs of DNA donor groups close to the optically active centres of the DNA molecules (but does not rule out the binding of other DNA donor groups). Low intensity signals are detected in the CD spectra in the 300–400 nm range (inset in Fig. 10); these correspond to electronic transitions of the metal complex upon its interaction with DNA groups. The

low intensity of the signal indicates that this interaction does not involve DNA atoms close to its chiral centres, and the possible donor atoms involved are from phosphate groups (see also below). The signals observed also change upon DNA degradation.

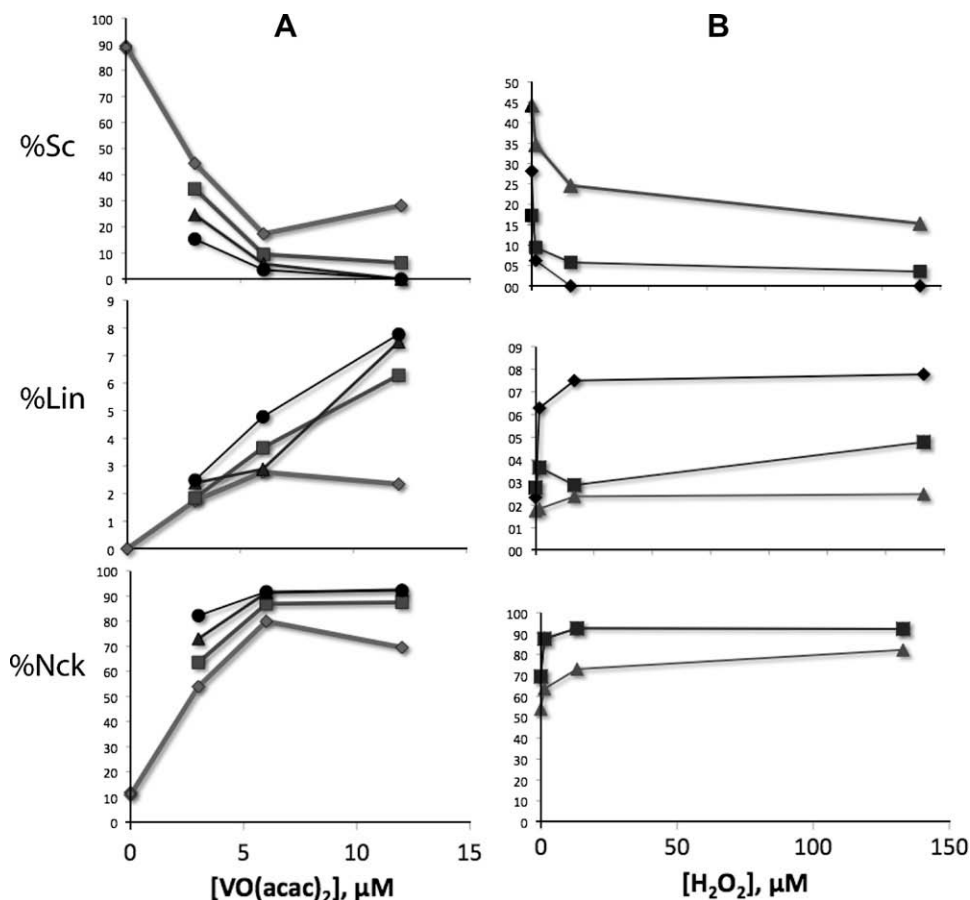
### 3.8. Effect of hydrogen peroxide on cleaving efficiency

The extent of plasmid degradation is higher in non-deoxygenated solutions, but the cleavage activity of **1** is also high in experiments under inert conditions. However, the concentration of complexes in the experiments is quite low and it is possible that very low amounts of  $O_2$  are present. Molecular oxygen activation in the presence of vanadium complexes may involve the oxidation of the metal centre with the production of superoxide radicals, which by dismutation produce  $H_2O_2$ . In the presence of the vanadium complexes this may yield hydroxyl radicals [50].

We examined the behaviour of **1** in the presence of physiological concentrations of  $H_2O_2$  (see Fig. 11). For example, experiments with ca. [12  $\mu M$  of **1** + 10  $\mu M$  of  $H_2O_2$ ] give similar cleavage as the use of 50  $\mu M$  of **1** and with ca. [3  $\mu M$  of **1** + 10  $\mu M$  of  $H_2O_2$ ] gave similar cleavage as the use of 12  $\mu M$  of **1**. This means that the presence of 10  $\mu M$   $H_2O_2$  enhances the pA1-cleavage by ~4-fold. Further increasing the concentration of  $H_2O_2$  does not lead to significant changes in the nuclease activity. Therefore, the DNA cleavage increases upon addition of  $H_2O_2$  but not in an intense manner.

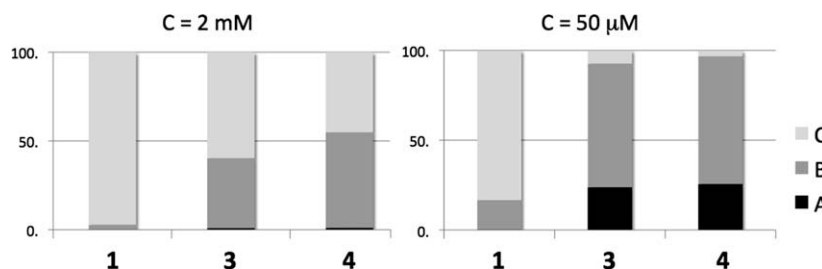
### 3.9. Mechanism and nature of the active species for DNA cleavage

The speciation of vanadium compounds in aqueous solutions may depend on the total vanadium concentration. It is known that



**Fig. 11.** Effect of  $H_2O_2$  on the nuclease activity of **1**. (A) Effect of complex concentration with constant peroxide content: (◆) no added  $H_2O_2$  (■) 1  $\mu M$  (▲) 10  $\mu M$  (●) 100  $\mu M$   $H_2O_2$ . (B) Effect of  $H_2O_2$  with constant complex concentration: (▲) 3  $\mu M$  (■) 6  $\mu M$  (◆) 12  $\mu M$   $V^{IV}O(acac)_2$ .





**Fig. 12.** Expected speciation of complexes **1**, **3** and **4** in aqueous solutions at pH 7 at millimolar and micromolar levels according to the published stability constants [32]. This does not take into account the possible oxidation of  $V^{IV}O^{2+}$  to  $V(V)$ . A:  $V^{IV}O^{2+}$ , B:  $V^{IV}OL^+$  and C:  $V^{IV}OL_2$ .

$V^{IV}O(acac)_2$  is reasonably stable towards oxidation in aqueous solutions [9,8,20]. Fig. 1 represents the theoretically possible structural isomers of complexes **1–4**. Two isomers are expected for **1** and **2**, either with a water molecule coordinated *cis* or *trans* relatively to the oxo group. X-ray crystallography of **1** shows only the *trans* isomer in the solid state. Experimental evidence by ENDOR [28] and EPR [30] points to the formation of only the *trans* isomer of  $V^{IV}O(acac)_2$  in solution, but some authors argue the *cis* form may also exist [9,7,32]. Regardless of whether the *cis* isomer is present or not (it may be present below the detection limit of experimental techniques), *trans* is undoubtedly the predominant form for these complexes.

The formation of the mono-chelated complex,  $V^{IV}OL^+$  has been reported in millimolar solutions by EPR at pH below 5.2 for **1** and 6.5 for **2** [30]. The DNA cleavage activity is obtained at micromolar levels and at such low concentrations the dissociation of the complex is expected to be more extensive. No suitable analytical methods are available to establish vanadium speciation at micromolar levels, and significant oxidation is also possible in these solutions. The actual species responsible for nuclease activity might not be the original neutral complex,  $V^{IV}OL_2$ ; a positively charged species, such as  $VOL^+$ , could be a better candidate as it would be attracted to the negative phosphate backbone of DNA. However,  $V^{IV}O(acac)_2$  and its derivatives can cleave DNA with remarkably different efficiencies. The fact that **1** and **2** are more stable complexes and have similar, much higher DNA cleavage efficiencies than **3** and **4** suggests that it is the neat complexes, and not the products of their decomposition, that have a relevant role in DNA degradation.

Based on stability constant data reported for complexes **1**, **3** and **4** [32], the distribution shown in Fig. 12 is expected at millimolar and micromolar levels. At a 50  $\mu M$ , **3** and **4** are mainly in the form of  $V^{IV}OL^+$ . As these are the least active complexes in DNA cleavage, it is reasonable to conclude that  $V^{IV}OL^+$  is not the active species.

The formation of the  $V^{IV}OL_2$  complexes is favoured in the presence of excess ligand. For **3** and **4**, this species is predominant at pH 6 when the ligand-to-metal ratio is 80 [32]. We tested the activity of **1**, **3** and **4** in the presence of excess ligand with L/M ratios between 2 and 324 (Fig. 9) and observed no increase in the nuclease activity of these complexes. Therefore, complex stability cannot be the only factor determining the different reactivities of these compounds.

Another possibility is that phosphate ions interact with  $V^{IV}O(acac)_2$ , for example by stabilizing the mono-chelated complex and facilitating its interaction with the DNA phosphate backbone. The interaction of  $V^{IV}O^{2+}$  and phosphate has been studied [51,52] but, to our knowledge, no speciation studies for  $VO(acac)_2$  in phosphate media have been reported. This has been done for maltolate, a small bidentate ligand with a stability similar to  $V^{IV}O(acac)_2$  [53]. Above pH 6, when the ratio metal:maltolate:phosphate is 1:5:5, phosphate cannot compete with maltolate for binding to vanadium. On the other hand, on serum medium, with phosphate con-

centration of 1 mM and metal concentration of 10  $\mu M$ , it was predicted that 8% of vanadium forms a binary complex with maltolate and phosphate. The stability constant of  $V^{IV}O(acac)_2$  is close to that of  $V^{IV}O(maltolato)_2$  (15.6 and 16.3), so we cannot exclude that a micromolar solution of  $V^{IV}O(acac)_2$  in a 10 mM phosphate buffer will also contain a fraction of the ternary complex  $V^{IV}O(acac)$ -(phosphate).<sup>5</sup> This species may contribute to the nuclease activity, but as discussed in Section 3.2.3 our available data suggests that it does not play a central role.

The nuclease activity of many inorganic complexes has been attributed to the formation of OH radicals by Fenton-type reactions [2]. Literature data indicate that vanadium compounds induce oxidative stress and free radical production is strongly implicated in the majority of anticancer effects of vanadium compounds, e.g., peroxidase activity of  $V(IV)$  compounds generates OH radicals leading to oxidative cleavage of DNA.

Our results show that  $V^{IV}O(acac)_2$  is clearly a more efficient nuclease in the presence of air or another oxidating agent like oxone or  $H_2O_2$ . Such a dependence points to a mechanism mediated by ROS. The detection and identification of short lived ROS can be elusive. Those most usually reported for oxidative cleavage by metalonucleases are hydroxyl radical and singlet oxygen [2]. Singlet oxygen is generally formed by photosensitization reactions [41]. We observed that the nuclease activity of **1** did not depend on photoirradiation, and so it is more likely caused by hydroxyl radicals than by  $^1O_2$  [41]. A mechanism involving the formation of OH radicals explains the quenching of the cleavage reaction whenever a radical scavenger is present. We could not confirm the formation of OH radicals by EPR spin trapping with DMPO, but our fluorimetric results with benzoate solutions confirmed there is a slow generation of hydroxyl radicals in aqueous solutions of  $VO(acac)_2$ .

In deoxygenated solutions, a lower but still significant nuclease activity was observed. This could be due to incomplete purging of  $O_2$  and/or oxygen impurities present in the nitrogen gas. Such low concentrations of oxygen would require the oxidative mechanism to be extremely sensitive to  $O_2$ . We have nevertheless observed by fluorometry that the formation of OH radicals by **1** in oxygenated solutions is considerably slower than when an excess of  $H_2O_2$  is added. We have also observed by gel electrophoresis that

<sup>5</sup> Our experiments show that solutions of ca. 6 mM **1** dissolved in ca 10% DMSO and 200 mM hepes (A) or 200 mM tris (B) have EPR spectra similar to solutions of **1** in DMSO. Trying to prepare the same solutions in 30% DMSO and 200 mM phosphate buffer (C) results in the precipitation of a small amount of a green solid, possibly a  $V(IV)$ -phosphate polymer, the EPR signal of the solution being much weaker than those of A and B. The EPR spectra of the three solutions are very similar, with peaks in the same positions. After complete oxidation under air for ca. 60 h, the  $^{51}V$  NMR spectra of solutions A and B are similar,  $V_1$ ,  $V_2$ ,  $V_4$  and  $V_5$  being the main species detected. The  $^{51}V$  NMR spectrum of solution C (also after ageing for 60 h) differs: it is much less intense and a weak broad signal at  $-562$  ppm is recorded. These results reveal a different behavior of **1** in phosphate buffer, and suggest the interaction of **1** with phosphate. Whether this is relevant or not for the mechanism of DNA cleavage is not clear. Further studies are presently being carried out.

the oxidative mechanism is not very sensitive to the concentration of oxidant, because increasing the concentration of H<sub>2</sub>O<sub>2</sub> has a much less significant effect on DNA degradation than increasing the complex concentration. So it is more likely that a different mechanism predominates in the experiments carried out under nitrogen atmosphere. This would not involve intermediate ROS and would possibly be caused by a direct interaction between the metal complex and DNA, as suggested by our CD results. The details of such a mechanism can only be, at this point, speculative. A possible mechanism would proceed through coordination to the phosphate moiety and hydrolysis of the phosphodiester bond. This is the mechanism followed by natural nuclease enzymes, using Ca<sup>II</sup>, Mg<sup>II</sup> or Zn<sup>II</sup> ions in their active centers. Nevertheless, only a few Co<sup>III</sup> and Cu<sup>II</sup> complexes are known to cleave DNA by attack at the phosphodiester bond [2]. The vanadyl ion is also a “hard” species, a strong Lewis acid and may bind to the phosphate backbone of DNA as well. It has been shown that V<sup>IV</sup>O<sup>2+</sup> can bind to diphosphate and triphosphate [54–56]. Moreover, there is EPR and ENDOR evidence [57] that V<sup>IV</sup>O<sup>2+</sup> binds to a phosphated nucleobase, guanosine-5'-monophosphate, with the chelated phosphate group binding equatorially. Infrared spectra also showed that coordination takes place through the oxygen atoms of the phosphate group in the solid complex of V<sup>IV</sup>O with D-ribose-5-phosphate [58]. Whether vanadium will cleave the phosphodiester in plasmid DNA or not is a relevant information that is not reported in the literature, although evidence for binding of the vanadyl ion to DNA through the phosphate backbone has also been presented by using IR spectroscopy and capillary electrophoresis [59]. However, we observed that the vanadyl ion by itself does not promote DNA cleavage, while V<sup>IV</sup>O(acac)<sub>2</sub>, on the other hand, does it very efficiently, so if cleavage at the phosphodiester linkage is occurring, the nature of the ligand is also relevant in this process.

#### 4. Conclusions

VO(acac)<sub>2</sub> is a very efficient DNA cleaving agent above 1 μM (ri = 0.1) being effective in linearizing pA1 DNA at ~10 μM (ri ~ 1) level. It requires no activating agents, no air and no irradiation to degrade DNA. The active species is neither monovanadate nor the mono-chelated species V<sup>IV</sup>OL<sup>+</sup>, and it is the bischelated complex, V<sup>IV</sup>OL<sub>2</sub> (L = acac), that is most probably directly or indirectly involved. The possibility of the formation of a ternary complex involving acac and phosphate in solution, or of a V-(acac)<sub>n</sub>- (phosphate ester)<sub>DNA</sub> (n = 1 or 2) cannot be ruled out. VO(hd)<sub>2</sub> **2** is slightly less efficient in linearizing pA1 DNA, while **3** and **4** are much less efficient cleavers.

The overall DNA cleavage mechanism could not be clarified and further work is ongoing. The presence of H<sub>2</sub>O<sub>2</sub>, O<sub>2</sub> or oxone enhances the cleavage activity. It is likely that more than one pathway is taking place: (a) oxidation in the presence of O<sub>2</sub>, involving ROS – probably OH radicals [50]; (b) hydrolytic degradation by direct interaction of V<sup>IV</sup>O(acac)<sub>2</sub> with DNA, possibly at the phosphodiester bonds.

The nature of the pH buffer is an important factor in the observation of the nuclease activity by agarose gel electrophoresis, and probably the most usual buffers (such as tris, hepes or mops in millimolar concentrations) act as radical scavengers and thus inhibit the DNA cleavage. The use of phosphate buffer allows a more extensive DNA damage because it does not act as radical scavenger, and in our opinion it should be preferred in experimental essays regarding nuclease activity.

#### 5. Abbreviations

AFM atomic force microscopy  
AGE agarose gel electrophoresis

Bz sodium benzoate  
DMPO 5,5-dimethylpyrroline-N-oxide  
ENDOR electron nuclear double resonance spectroscopy  
hepes 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid  
Lin linear form of plasmid DNA  
mes 2-(N-morpholino)ethanesulfonic acid  
mops 3-(N-morpholino)propanesulfonic acid  
MPA mercaptopropionic acid  
Nck nicked (open circular) form of plasmid DNA  
PBS phosphate buffered saline  
phen 1,10-phenanthroline  
PO4 phosphate buffer  
ROS reactive oxygen species  
Sc supercoiled form of plasmid DNA  
tris tris(hydroxymethyl)aminomethane  
UV-vis UV-visible  
V1 monovanadate  
V2 divanadate  
V4 tetravanadate  
V5 pentavanadate  
V10 decavanadate

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#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.jinorgbio.2009.01.003](https://doi.org/10.1016/j.jinorgbio.2009.01.003).

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