Assessment of DNA binding mode, chemical nuclease activity and docking studies of novel vanadyl complexes

Poonam R. Inamdar\textsuperscript{a}, A. S. Vickram\textsuperscript{b}, T. B. Sridharan\textsuperscript{b} and A. Sheela\textsuperscript{a}

\textsuperscript{a}Materials Chemistry Division, School of Advanced Sciences, VIT University, Vellore-632 014, Tamilnadu, India
\textsuperscript{b}Industrial Biotechnology Division, School of Biosciences and Technology, VIT University, Vellore-632 014, Tamilnadu, India

Abstract: Transition metal complexes have been exploited extensively to design new drugs due to their favorable electronic environment and tunable geometry. These specific properties have proved to be advantageous for their versatile biological applications. Herein, we are focusing on synthesis of two ester based oxovanadium complexes, say, bis(hutanoic acid-3-oxo-1,1-dimethylethyl ester)oxovanadium(IV) (1) and bis(dipropan-2-yl-propenedioate)oxovanadium(IV) (2). Complexes were characterized by UV spectroscopy, \textsuperscript{\textgamma}TIR studies and Mass spectroscopic analysis. The complexes are assessed for chemical nuclease activity against plasmid pBR322 using gel electrophoresis. Further support towards binding mode is carried out by UV titration using calf thymus DNA. Docking studies are done to compare experimental and theoretical results.

Keywords: Oxovanadium complexes, DNA binding, nuclease activity, docking.

Introduction

Since the decades, transition metal complexes have played a crucial role in medicinal field. A variety of metal complexes have been reported for their antimicrobial, antimalarial, anticancer and antidiabetic potentials. The term ‘Elemental Medicine’ has been coined after successful emergence of ‘Cisplatin’ as anticancer agent. These complexes have pioneered the metal originated chemotherapy in cancer treatment, but severe side effects such as hepatotoxicity, nephrotoxicity have limited their use. This necessitates the chemists to search for new metal based drugs and recently vanadium is one such element studied towards this application.

Vanadium, being an essential trace element, had been focused for wide range of biological activities. Oxovanadium complexes have been studied extensively towards their insulin mimetic activity. Maltol based vanadium complex is an important metallodrug reported to have completed phase II clinical trials. In addition, vanadium complexes have also been probed towards their nuclease activity. As per the recent report, chemo-preventive activity in carcinomas\cite{1} has been discussed in detail. The prototype diketone based oxovanadium complex [\(\text{VO}^{IV}(\text{acac})_2\)] has arrested cell growth and blocked the cell cycle at G1 phase of HepG2 cells\cite{2}. In the similar way, phenanthroline and hydroxysalen based \(\text{V}^{IV}\) complexes and vanadium salts (\(\text{VOSO}_4\)) are considered to be efficient inorganic nuclease in the presence of activating agent like hydrogen peroxide\cite{3}, mercaptopropionionic acid\cite{4} etc. Recently in 2009, Butenko\cite{5} reported four different diketone based vanadium complexes for their nuclease activity and has highlighted upon the reactive species responsible for DNA cleavage. In the current study, we have explored the oxidative DNA cleavage and binding mode of ester based oxovanadium complexes monitored through gel electrophoresis and UV-Visible titration methods respectively. Further, insilico studies are also carried out to correlate the results with experimental observations.

Experimental

\textbf{Materials and methods}:

Tert-butyl-3-oxobutanoate, dipropan-2-yl-propanedioate (Sigma-Aldrich), vanadium pentoxide (Thomas
Baker) and CT-DNA, pUC 19 (Merck) are procured and used without purification. Electronic spectra-JASCO UV-VIS-NIR-V-670. FTIR spectra (KBr pellets)-Shimadzu IR affinity-1CE model with resolution 4. Mass spectra was recorded on Shimadzu QP 5000 spectrometer.

Synthesis of oxovanadium complexes:
Oxovanadium complexes of ester based complexes are synthesized in two steps, the first step being reduction of \( \text{V}_2\text{O}_5 \) in presence of \( \text{H}_2\text{SO}_4 \) and ethanol. In the next step, it is added to esters in 1:2 molar ratios and refluxed for 2 h. The resultant mixture is neutralized by \( \text{K}_2\text{CO}_3 \) along with stirring.

UV absorption titration:
Absorption titration was carried out in presence of constant complex concentration (20 \( \mu \text{M} \)) by varying CT-DNA concentration from 20-140 \( \mu \text{M} \). All the readings are recorded using Tris HCl buffer (pH 7.2).

DNA cleavage:
Cleavage experiments are carried out by gel electrophoresis on pUC 19 by complexes (80 \( \mu \text{M} \)) in TAE buffer. The samples are incubated for 2.5 h at 37 \( ^\circ \text{C} \). Loading dye, Bromophenol blue is added and electrophoresis was carried out at 50 V using 0.8% agarose containing 1 \( \mu \text{g/mL} \) ethidium bromide. The cleavage is monitored in the presence and absence of the activating agent, hydrogen peroxide. The gel, after completion was visualized under UV illumination and the image is captured.

Molecular docking studies:
Autodock Vina\(^9\) is used to perform docking studies of synthesized complexes using DNA sequence PDB ID: 423D. Ligand (complex) and receptor (DNA) molecules are prepared using Autodock tools 4.2. DNA is enclosed in specific grid points. The lowest energy conformer of each complex is selected and used to study the interaction.

Results and discussion

Synthesis and characterization:
Complexes 1 and 2 are synthesized by direct reaction between vanadium sulphate, reduced from vanadium pentoxide and corresponding esters in molar ratio 1:2, in presence of \( \text{K}_2\text{CO}_3 \). The complexes are synthesized in good yields and recrystallized. The absorption spectra of these complexes are typical as intense UV bands are assignable to intraligand \( \pi-\pi^* \) transitions at 258 nm for complex 1 and 242 nm for complex 2. The absorption bands observed in visible region as broad peak at 520 nm for complex 1 and 690 nm for complex 2 respectively are due to d-d transitions. FTIR data shows that \(-\text{C}=\text{O}\) stretch in tert-butyl 3-oxobutanato (ligand 1) is observed at 1722.17 cm\(^{-1}\) which is shifted to lower wave number at 1635.64 cm\(^{-1}\) in complex 1. Similarly, \(-\text{C}=\text{O}\) stretch in dipropan-2-yl propanedioate (ligand 2) is observed at 1729.09 cm\(^{-1}\) which is also shifted to 1622.13 cm\(^{-1}\) after complexation to vanadium metal. The typical V=O bond stretching frequencies occur at 996.27 cm\(^{-1}\) and 975.98 cm\(^{-1}\) for complex 1 and 2 respectively which are not observed in the ligand IR spectra. Mass spectrum of complex 1 shows the base peak at \( m/z \) 381.3656 and for complex 2 at \( m/z \) 446.2328.

UV absorption titration for DNA binding study:
UV-Visible absorption spectra of complexes 1 and 2 are recorded in water in the presence of increasing concentrations of DNA depicted in Fig. 1. When compared with the UV bands of complexes in the absence of DNA, it has been observed that on binding to DNA, intraligand bands in UV region are perturbed. In case of both the complexes, as DNA concentration was increased successively at \( \lambda_{\text{max}} \) 265 nm for complex 1 and at 254 nm for complex 2, strong hyperchromic shift was observed in both the complexes suggesting groove binding mode\(^6\).

DNA cleavage experiment:
As UV absorption titration predicts groove binding of complexes to DNA, the nuclease activity of synthesized complexes done by gel electrophoresis measures the ability of complexes to convert supercoiled DNA (Form I) to nicked circular (Form II) or linear circular (Form III)\(^7\) as depicted in Fig. 2. Nuclease efficiency depends on the use of activators which is mixed with complex and DNA before incubation. Hydrogen peroxide is the preferred activating agent.

Based on the results obtained, it is found that complexes 1 and 2 are able to cleave DNA in Form II (nicked circular) and Form III (linear circular) as shown in Fig. 2. The complexes do not show remarkable cleaving ability in the absence of \( \text{H}_2\text{O}_2 \) as shown in Lane 3 and 5, but behaves as an efficient inorganic nuclease in presence of \( \text{H}_2\text{O}_2 \), as observed in Lane 4 and 6 (Fig. 2).
Molecular docking of complexes with DNA:
In order to further investigate the binding interactions of these complexes, docking studies are carried out with DNA (PDB ID: 423D)\textsuperscript{7}. The minimum energy conformer of complexes 1 and 2 show both covalent and non-covalent interactions with DNA (Fig. 3). The complexes bind to DNA groove preferably, thus, correlating with the results obtained from UV titration method.

Conclusion
Two ester based oxovanadium complexes have been synthesized and characterized. Based on the results of UV titration method, the possible binding mode is predicted to be groove binding. The gel electrophoretic study shows that the cleaving ability of the complexes is improved by the use of activators. The results of docking studies also confirm the favorable groove binding interactions with DNA thus bringing about the correlation between theoretical predictions and experimental results.

References