Journal of Photochemistry & Photobiology, B: Biology 160 (2016) 110–120

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Synthesis, interactions, molecular structure, biological properties and molecular docking studies on Mn, Co, Zn complexes containing acetylacetone and pyridine ligands with DNA duplex

V. Thamilarasan [a], N. Sengottuvelan [b,*], N. Stalin [c], P. Srinivasan [c], G. Chakkaravarthi [d]

[a] Department of Industrial Chemistry, Alagappa University, Karaikudi 630 003, India
[b] Department of Chemistry, DDE, Alagappa University, Karaikudi 630 003, India
[c] Department of Bioinformatics, Alagappa University, Karaikudi 630 004, India
[d] Department of Physics, CPCL Polytechnic College, Chennai 600 068, India

ARTICLE INFO

Article history:
Received 31 July 2015
Received in revised form 20 February 2016
Accepted 14 March 2016
Available online 18 March 2016

Keywords:
Mn^{II}
Co^{II}
Zn^{II} complexes
DNA/protein interaction
Molecular modeling
Cytotoxic activities

ABSTRACT

Three metal complexes (**1**–**3**) of the type $[Mn(acac)_2(py)\cdot H_2O]$ (**1**), $[Co(acac)_2(py)\cdot H_2O]$ (**2**) and $[Zn(acac)_2(py)\cdot H_2O]$ (**3**), [Where acac = acetylacetone, py = pyridine] were synthesized and characterized by spectral (UV–vis, FT-IR, ESI-mass) analysis. The structure of complex **2** has been determined by single crystal X-ray diffraction studies and the configuration of ligand-coordinated to metal(II) ion was well described as distorted octahedral coordination geometry. The interaction of the complexes with CT-DNA has been explored by absorption, fluorescence, circular dichromism spectroscopy, viscosity measurements and molecular docking studies. The intrinsic binding constant K_b of complexes **1**–**3** with CT-DNA obtained from UV–vis absorption spectral studies were 2.1×10^4, 2.1×10^5 and 1.98×10^4 M^{-1}, respectively, which revealed that the complexes could interact with CT-DNA through groove binding. The results indicated that the complexes (**1**–**3**) were able to bind to DNA with different binding affinity, in the order: **2** > **1** > **3**. The interaction of the compounds with bovine serum albumins were also investigated using fluorescence methods and the gel electrophoresis assay demonstrates weak cleavage ability of the pBR322 plasmid DNA in the presence of the metal complexes (1–3) with various activators. Further, the in vitro cytotoxic effect of the complexes were examined on cancerous cell line, with human breast cancer cells MCF-7.

1. Introduction

The coordination chemistry of transition metal complexes with mixed ligands is of current interest because they can provide new materials with useful properties such as antifungal, antibacterial, anticancer properties [1,2], magnetic exchange [3,4], electrical conductivity [5], photoluminescence [6], and nonlinear optical property [7]. The biological importance of mixed ligand complexes is that they are sometimes more effective than the free ligands [8]. Metal complexes containing pyridine and derivatives have aroused considerable interest in view of their industrial and biological importance [9,10]. Many of these compounds possess a wide spectrum of medicinal properties, including activity against tuberculosis, bacterial and viral infections. They have also been found to be active against influenza, certain kinds of tumors and have been suggested as possible pesticides and fungicides. Their activity has frequently been thought to be their ability to chelate trace metals [11–13]. The transition metal β-diketone compounds were used extensively as starting materials in the early days of metallocene chemistry [14], and as electroluminescent materials [15,16]. Also it has been found to possess fungicidal and insecticidal activities [17]. Recently, applications of these transition metal complexes in the design and development of synthetic restriction enzymes, new drugs and stereoselective probes of nucleic acids structure have been explored extensively [18]. Transition metal complexes offer two peculiar advantages as DNA-binding agents [19]. First and foremost, transition metal centers are particularly attractive moieties for reversible recognition of nucleic acids research because they exhibit well-defined coordination geometries. Moreover, they often possess distinctive electrochemical or photophysical properties, thus enhancing the functionality of the binding agent [20]. Indeed, these characteristics have prompted metal complexes to be used in a wide range of applications, from fluorescent markers to DNA footprinting agents, to electrochemical probes [21].

Recently, we reported the synthesis and characterization of Mn(II), Co(II) and Zn(II) complexes containing 2,2′-bipyridine-azide mixed ligands. Particularly, in the case of Co(II) with planar aromatic bipyridine-azide mixed ligand complex characteristic data show them to have higher DNA binding, cleavage and cytotoxic activity compared to the similar

* Corresponding author at: Department of Chemistry, DDE, Alagappa University, Karaikudi 630 003, India.
E-mail addresses: nsvelan1975@yahoo.com, nsvelan1975@yahoomail.com (N. Sengottuvelan).

http://dx.doi.org/10.1016/j.jphotobiol.2016.03.018
1011-1344/

Mn(II) and Zn(II) complexes [22]. We also reported the biological activity of planar aromatic 8-hydroxyquinoline based distorted octahedral Mn(III) complex [23]. Since aromatic moieties are known to play an important role in enhancing DNA binding and cleavage activity. In continuation of our ongoing research work on metal-DNA interactions, here in report the acetylacetone-pyridine based mixed ligand complexes i.e., $[Mn(acac)_2(py)\cdot H_2O]$ (**1**), $[Co(acac)_2(py)\cdot H_2O]$ (**2**) and $[Zn(acac)_2(py)\cdot H_2O]$ (**3**) and their structural studies, DNA/protein binding, cleavage properties and anticancer activity.

2. Experimental Section

2.1. General

Manganese chloride, Cobaltous chloride, Zinc chloride, Ethidium bromide (EB), Bovine serum albumin (BSA), Calf thymus DNA (CT-DNA) were purchased from Sigma-Alrich (India) and acetylacetone and pyridine (SRL) were used as received. The solvents used were of reagent grade. UV–vis spectrophotometer was employed to check DNA purity (A_{260}: $A_{280} > 1.80$) and concentration ($e = 6600\ M^{-1}\ cm^{-1}$ at 260 nm). Double distilled water was used to prepare buffers.

2.2. Physical Measurements

The mass spectra of the complexes were determined at a Shimadzu QP 2000 mass spectrometer, SAIF, Lucknow, India. Elemental analyses of carbon, hydrogen, and nitrogen were performed on a Carlorerba-1106 microanalyzer. The electronic spectra were recorded using a Shimadzu UV-3101PC spectrophotometer. FT-IR spectra were recorded in the 4000–400 cm^{-1} region using KBr pellets by a Bruker EQUINOX 55 spectrometer. The fluorescence study was carried out using an Elico SL 174 spectrofluorometer. The molar conductivity of a freshly prepared solution of the complexes (10^{-3} M) in DMF was measured using an Elico Model SX80 conductivity bridge. The CD spectra were recorded using Jasco J-810 spectropolarimeter.

2.3. Crystal Structure Determination and Refinement for Complex 2

The X-ray diffraction measurements were made using a Bruker APEX II CCD area detector diffractometer (Mo Kα radiation, graphite monochromator, λ = 0.71073 Å) and needle-shaped black crystals of complex **2** with dimensions $3.50 \times 0.30 \times 0.25\ mm^3$, chosen and mounted on a glass fiber and used for data collection. Cell constants and an orientation matrix for data collection were obtained by least square refinements of diffraction data from 2016 unique reflections. Data were collected at room temperature with a maximum 2θ value of 56.44° in a series of Ω scans in 1° oscillations. Semi-empirical absorption corrections were carried out using the program SADABS [24]. The structures were solved by direct methods using the program SHELXS-97. The refinement and all further calculations were carried out using SHELXL-97. The C-bound H-atoms were included in calculated positions and treated as riding atoms using SHELXL-97 default parameters. The non-H atoms were refined anisotropically, using weighted fullmatrix least-squares on F^2. Software packages APEX2 (data collection), SAINT (cell refinement and data reduction) and SHELXTL (molecular graphics and publication material) were also used [25,26].

2.4. Synthesis of Complexes

2.4.1. $[Mn(Acac)_2(Py)\cdot H_2O]$ (1)

Pyridine (0.1 g, 1.26 mmol) in methanol (10 mL) was mixed with sodium hydroxide (0.05 g, 1.26 mmol) and the resulting solution was stirred for 15 min at room temperature. Further, manganese chloride (0.25 g, 1.26 mmol) in methanol (10 mL) was added in to the solution. To the reaction mixture acetylacetone (0.13 g, 1.26 mmol) was added followed by stirring for 3 h during which a dark brown precipitate was formed. Yield: 48%; m.p. 149 °C (dec). Anal. Calc. (%) for $C_{15}H_{20}MnNO_5$: C, 51.42; H, 6.00; N, 4.00. Found (%): C, 51.16; H, 6.14; N, 3.77. FT-IR, (ν, cm^{-1}) (KBr Disk): 3413br, 1642s, 1605s, 1572s. UV–vis in DMF [λ_{max}/nm (ε_{max}/$mol^{-1}\ cm^{-1}$)]: 269(1454), 342(448), 641(1160). ESI-MS (CH_3CN) *m/z* (%): 348(15) $[Mn(acac)_2(py)\cdot H_2O]$, 346(40)(m-2) $[Mn(acac)_2(py)\cdot H_2O]$. Conductivity ($\Lambda_M$/$Scm^2\ mol^{-1}$) in DMF: 12.

2.4.2. $[Co(Acac)_2(Py)\cdot H_2O]$ (2)

The complex **2** was obtained using the same procedure as **1** using cobaltous chloride (0.30 g, 1.26 mmol) instead of manganese chloride. The resulting black colored precipitate that formed was dissolved in DMF and kept for crystallization. Four days later needle-shaped black color crystals suitable for X-ray diffraction were obtained. Yield: 58%; m.p. 154 °C (dec). Anal. Calc. (%) for $C_{15}H_{20}CoNO_5$: C, 50.84; H, 5.93; N, 3.95. Found (%): C, 50.58; H, 6.07; N, 3.72. FT-IR, (ν, cm^{-1}) (KBr Disk): 3433br, 1618s, 1571s. UV–vis in DMF [λ_{max}/nm (ε_{max}/$mol^{-1}\ cm^{-1}$)]: 266(511), 417(172), 607(24), 671(27). Conductivity (Λ_M/$Scm^2\ mol^{-1}$) in DMF: 15.

2.4.3. $[Zn(Acac)_2(Py)\cdot H_2O]$ (3)

The complex **3** was also obtained from the same procedure as described above using zinc chloride (0.17 g, 1.26 mmol) instead of manganese chloride. The yellow colored precipitate was formed. Yield: 57%; m.p. 161 °C (dec). Anal. Calc. (%) for $C_{15}H_{20}ZnNO_5$: C, 50.13; H, 5.84; N, 3.89. Found (%): C, 49.87; H, 5.98; N, 3.66. FT-IR, (ν, cm^{-1}) (KBr Disk): 3417br, 1635s, 1602s, 1568s, 1518s. UV–vis in DMF [λ_{max}/nm(ε_{max}/$mol^{-1}\ cm^{-1}$)]: 280(2283), 345(3883), 408(1144). ESI-MS (CH_3CN) *m/z* (%): 359(25) $[Zn(acac)_2(py)\cdot H_2O]$, 293(59) $[(acac)_2(py)\cdot H_2O]$, 277(73) $[Zn(acac)_2\cdot H_2O]$, 192 (90) (m-2) [Zn(acac)]. Conductivity (Λ_M/$Scm^2\ mol^{-1}$) in DMF: 11.

2.5. DNA Binding Studies

2.5.1. Absorption Spectrophotometric Studies

Absorption spectra titrations were performed at room temperature in Tris HCl/NaCl buffer (5 mM HCl/50 mM NaCl buffer, pH 7.2) to investigate the binding affinity between CT-DNA and complexes. Electronics spectra of the complexes were recorded before the addition of DNA. The intrinsic binding constant for the interaction of complexes with DNA was obtained from absorption data. A fixed concentration of the complexes was titrated with increasing amounts of DNA concentration.

2.5.2. Competitive Binding Experiments

The relative binding of complexes to CT-DNA were determined with an EB-bound CT-DNA solution in Tris–HCl/NaCl buffer (pH 7.2, 5 mM Tris–HCl/50 mM NaCl). The influence of the addition of complexes to the EB-DNA complexes have been obtained by recording the variation of fluorescence emission spectra with excitation at 510 nm and emission at 600 nm.

2.5.3. CD Spectrophotometric Studies

The CD spectra of CT-DNA in the absence or presence of complexes were collected after 12 h incubation of 100 μM CT-DNA with 50 μM individual complexes in Tris–HCl buffer (pH 7.2) containing 5 mM/50 mM NaCl at 37 °C. For each spectrum, three scans were collected and the background was subtracted from all of the reagents by using a corresponding solution without CT-DNA as a reference solution. All CD experiments were performed on a Jasco J-810 spectropolarimeter at room temperature from 320 to 220 nm.

2.5.4. Viscosity Measurements

The viscosity of solution relied on the time flowed through the capillary viscometer. The viscosity measurements were conducted by keeping the viscometer immersed in a water-bath maintained at 25 °C ± 0.1 °C. Flow times were measured and the relative viscosity (g) was obtained by the equation: $\eta = (t - t_0) / t_0$, where t and t_0 represent the observed flow time of CT-DNA containing solutions and buffer solution alone,