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DNA binding/cleavage, antioxidant and cytotoxic activities of water soluble cobalt(II) and copper(II) antipyrine complexes

Subbaiyan Sathiyaraj [a], Krishnan Sampath [a], Gunasekaran Raja [a], Ray J. Butcher [b], Sushil K. Gupta [c], Chinnasamy Jayabalakrishnan [a,*]

[a] Post Graduate and Research Department of Chemistry, Sri Ramakrishna Mission Vidyalaya College of Arts and Science, Coimbatore 641 020, Tamil Nadu, India
[b] Department of Chemistry, Howard University, Washington, DC 20059, USA
[c] School of Studies in Chemistry, Jiwaji University, Gwalior 474 011, Madhya Pradesh, India

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ABSTRACT

Two transition metal complexes have been synthesized by reacting antipyrine (AP) with $CoCl_2{\cdot}6H_2O$ or $CuCl_2{\cdot}2H_2O$. The structures of the complexes have been determined by single crystal X-ray diffraction studies. The interaction of cobalt(II) and copper(II) complexes with calf thymus DNA (CT-DNA) was investigated by electronic absorption spectroscopic technique. The experimental evidences indicated that the two water-soluble metal(II) complexes could strongly bind to CT-DNA *via* intercalation mechanism. A gel electrophoresis assay demonstrated the ability of the complexes to cleave the pBR322 plasmid DNA *via* oxidative pathway. Investigation of the antioxidative properties showed that the two water soluble metal(II) complexes have a significant radical scavenging potency against DPPH, OH and ABTS radicals. Further, the cytotoxic effect of the compounds examined on cancerous cell lines, viz., HeLa, HEp-2 and MCF-7, showed that the complexes exhibited substantial cytotoxic specificity on HeLa over the other two cell lines.

1. Introduction

The interaction of transition metal complexes with DNA has been a subject of passionate research in the field of bioinorganic chemistry, ever since the discovery of cis-platin as an anticancer agent. As an important intention of anticancer drugs, DNA plays a central role in replication, transcription, and regulation of genes. The presence of metal-binding sites in DNA structure make different type of interactions possible such as intercalation between base pairs, minor groove binding, and major groove binding [1]. However clinical drawbacks of cis-platin, which include acquired conflict and the limited spectrum of the anticancer activity, gave an impetus to develop alternative transition metal complexes which are less toxic and more effective for chemotherapeutic application [2–7]. Additionally, it has been demonstrated that free radicals can damage proteins, lipids, and DNA of bio-tissues, leading to increased rates of cancer [8]. Fortunately, antioxidants can prevent this damage, due to their free radical scavenging activity [9]. Hence, it is very important to develop compounds with both strong antioxidant and DNA-binding properties for effective cancer therapy.

This inspires synthetic chemists to search for new metal complexes for bioactive compounds, transition metals are particularly suitable for this purpose because they can adopt a wide variety of coordination numbers, geometries and oxidation state in comparison with carbon and other main group elements. Above all, copper and cobalt has attracted the researchers. Probably the most widely studied cation in this respect is Cu^{2+}, since a host of low-molecular-weight copper complexes have been proven beneficial against several diseases such as tuberculosis, rheumatoid, gastric ulcers and cancers [10]. It was reported that, the treatment with the copper complexes produce remarkable pharmacological effects, which are not observed when the parent ligands or inorganic forms of copper are used [11]. Cobalt complexes have gained significance as the recent studies have shown that it can produces more active oxygen species that may be associated with the induction of chromosomal aberrations and mutation [12].

Pyrazolones are a class of organic compounds that have been studied extensively due to their pharmaceutical properties. Pyrazolone is a five-membered lactam ring which contains two nitrogens and a ketone in the same molecule, and is an active moiety in pharmacological activity, such as antiflammatory agents [13], for the treatment of arthritis [14] and as analgesics [15]. Anticancer activity has also been reported [16]. Pyrazolones have also found applications outside the pharmaceutical field, such as in the solvent extraction of metal ions [17], for analytical purposes [18]

* Corresponding author. Tel.: +91 4222692461.
E-mail address: cjayabalakrishnan@gmail.com (C. Jayabalakrishnan).

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and as ligands in complexes with catalytic activity [19]. From a coordination chemistry viewpoint, the only atoms available for coordination are the nitrogen atoms of the pyrazole ring and the oxygen atom of the carbonyl group. If the nitrogens are blocked by substitution, such as in antipyrine, coordination can only be achieved through the oxygen atom. Considerable current interest in nucleic acid chemistry is now directed to copper(II) and cobalt(II) complexes containing heterocyclic bases, due to their diverse applications. So here in, we report the synthesis, structure and biological evolution of a novel water soluble cobalt(II) and copper(II) antipyrine complexes.

2. Experimental

2.1. Materials and instrumentation

Reagent grade chemicals were used without further purification in all the synthetic work. All the solvents were purified by standard methods [20]. $CuCl_2{\cdot}2H_2O$, $CoCl_2{\cdot}6H_2O$ and antipyrine were purchased from Himedia Company and used as received. Calf thymus DNA (CT-DNA) and pBR322 DNA were purchased from Bangalore Genei, Bangalore, India. The Human Cervical cancer cell lines (HeLa), human laryngeal epithelial carcinoma cell line (HEp-2) and human breast cancer cell line (MCF-7) were obtained from National center for cell science (NCCS), pune, India.

Infrared spectra were recorded on a FT-IR Perkin Elmer spectrophotometer RXI model as KBr pellets in the range 4000–400 cm^{-1}. Elemental analyses were performed with a model Vario ELIII CHNS at Sophisticated Test and Instrumentation Centre (STIC), Cochin University, Kerala. Electronic spectra were recorded in DMSO solution in a Systronics 2202 Double beam spectrophotometer in 800–200 nm range. DNA cleavage studies were carried out using Gelstan, Gel documentation system. Antioxidant and anticancer studies were carried out at the Kovai Medical Centre and Hospital Pharmacy College, Coimbatore, Tamilnadu. Melting points were recorded with a Veego DS model apparatus and are uncorrected.

2.2. Synthesis of metal(II) complexes

Complexes $[CoCl_2(AP)_2]$ and $[CuCl_2(AP)_2]$ were synthesized by refluxing 1:2 amount of appropriate metal salts $CoCl_2{\cdot}6H_2O$ (0.237 g; 1 mmol) or $CuCl_2{\cdot}2H_2O$ (0.170 g; 1 mmol) with the antipyrine (AP) (0.376 g; 2 mmol) in ethanolic solution for 2 h (Scheme 1). Blue and red colored crystals suitable for X-ray studies were obtained on slow evaporation of the reaction mixture in both the cases. They were filtered off, washed with cold ethanol and dried under vaccum.

The data corresponding to complex $[CoCl_2(AP)_2]$ were as follows: Yield: 78%. mp: 118 °C. *Anal.* Calc. for $C_{22}H_{24}Cl_2CoN_4O_2$: C, 52.19; H, 4.78; N, 11.07. Found: C, 52.38; H, 4.83; N, 11.12%. Selected IR bands (ν in cm^{-1}): 1632 (C = O pyrazolone ring). UV–Vis (in Ethanol); λmax, nm; 267, 651.

The data corresponding to complex $[CuCl_2(AP)_2]$ were as follows: Yield: 78%. mp: 120 °C. *Anal.* Calc. for $C_{22}H_{24}Cl_2CoN_4O_2$: C, 51.72; H, 4.73; N, 10.97. Found: C, 51.64; H, 4.59; N, 10.84%. Selected IR bands (ν in cm^{-1}): 1628 (C=O pyrazolone ring). UV–Vis (in DMSO); λmax, nm; 262, 294, 361, 390.

2.3. Crystallography

Single crystal X-ray diffraction data of $[CoCl_2(AP)_2]$ and $[CuCl_2(AP)_2]$ were collected at room temperature on a Bruker Kappa Apex II CCD diffractometer equipped with a fine focused sealed tube. The unit cell parameters were determined and the data collections of the complexes were performed using a graphite-mono chromate Mo Kα (λ = 0.71073 Å) radiation by φ and ω scans. The data collected were reduced SAINT program [21] and the empirical absorption corrections were carried out using the SADABS program [22]. The structure of the complexes was solved by direct methods [23] using SHELXS-97, which revealed the position of all non-hydrogen atoms, and was refined by full-matrix least squares on F^2 (SHELXL-97) [23]. All non-hydrogen atoms were refined anisotropically, while the hydrogen atoms were placed in calculated positions and refined as riding atoms.

2.4. DNA binding – titration experiments

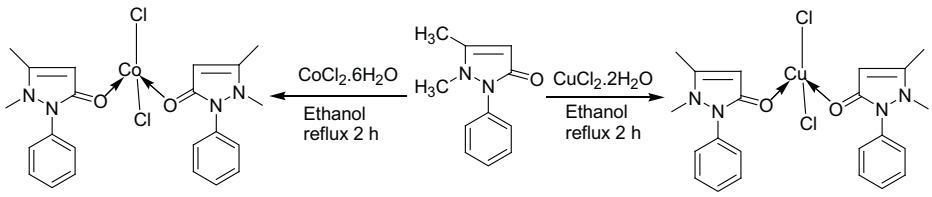
Experiments involving the interaction of cobalt(II) and copper(II) complexes with CT-DNA were carried out in double distilled water with tris(hydroxymethyl)-aminomethane (Tris, 5 mM) and sodium chloride (50 mM) and adjusted to pH 7.2 with hydrochloric acid. A solution of CT-DNA in the buffer gave a ratio of UV absorbance of about 1.9 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar extinction coefficient value of 6600 $dm^3\ mol^{-1}\ cm^{-1}$ at 260 nm. Electronic absorption titration experiments were performed by maintaining the concentration of the complex as constant (25 μM) but with variable nucleotide concentration from 0 to 25 μM. While measuring the absorption spectra, equal amounts of DNA were added to both compounds and reference solutions to eliminate the absorbance of DNA itself. The data were then fit into the following equation and the intrinsic binding constant K_b was calculated in each case [24].

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/k_b(\varepsilon_b - \varepsilon_f)$$

where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficient ε_a, ε_f and ε_b correspond to A_{obsd}/[complex], the extinction coefficient of the free compound and the extinction coefficient of the compound when fully bound to DNA respectively. In plots of $[DNA]/(\varepsilon_a - \varepsilon_f)$ versus [DNA], K_b is given by the ratio of slope to the intercept.

2.5. DNA cleavage experiment

The DNA cleavage activity of the cobalt(II) and copper(II) complexes was monitored by agarose gel electrophoresis on pBR322 DNA. The tests were performed in the absence and presence of activating agent, H_2O_2 under aerobic condition. Hydrolytic cleavage was monitored by 30 μM of pBR322 DNA and 30; 60 μM of



Scheme 1. Synthesis of cobalt(II) and copper(II) antipyrine complexes.