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Synthesis and characterization of novel square planar copper(II)–dipeptide–1,10-phenanthroline complexes: Investigation of their DNA binding and cleavage properties

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ABSTRACT

In view of the importance of aromatic moieties in DNA binding and cleavage, two new Cu(II)–dipeptide–phen (phen = 1,10-phenanthroline) complexes, viz. [Cu(II)(boc(tos)-his-trp-ome)(phen)]$(ClO_4)_2$ (**1**) and [Cu(II)(boc(tos)-his-tyr-ome)(phen)]$(ClO_4)_2$ (**2**) were synthesized, comprehensively characterized and their structures reported. The physico-chemical data and molecular modeling approaches support that the complexes are arranged in a distorted square planar geometry. To provide an insight on the mode and affinity of the interaction of these complexes with calf thymus (CT) DNA the following experiments were carried out: thermal denaturation (T_m), UV–Vis absorption, competitive binding, viscosity and fluorescence spectroscopy studies. These experimental results indicate that the complexes interact through stacking between the base pairs of double helix DNA. The overall binding constants (K_b) were determined as 3.33×10^4 and 2.82×10^4 M^{-1} for **1** and **2** respectively. The complexes converted supercoiled plasmid pUC19 DNA (SC DNA) into its nicked (NC) form both in the absence (hydrolytic cleavage) and presence (oxidative cleavage) of H_2O_2. The control experiments demonstrate that no diffusible radical species were involved in the hydrolytic cleavage, but in the presence of H_2O_2, hydroxyl radical ($OH^{\cdot}$) species were generated and initiated the cleavage reaction by an oxidative pathway. The rate constants for the hydrolysis of the phosphodiester bond were determined as 1.70 h^{-1} and 1.82 h^{-1} for **1** and **2** respectively. This amounts to a $(4.7–5.0) \times 10^7$-fold rate enhancement compared to non-catalyzed DNA cleavage, which is significant. These complexes exhibit better DNA binding and cleavage abilities compared to similar reported complexes.

1. Introduction

The design of artificial nucleases represents an area of substantial interest since nucleases have wider applications in molecular biology in probing the structures and functions of nucleic acids [1–4]. In contrast to natural DNA-binding enzymes, low molecular artificial nucleases exhibit higher stability and less reactive condition dependence when they are used as an accelerator in DNA cleavage [5,6].

Many studies suggest that DNA is an important primary cellular receptor. Many chemicals exert their antitumor effects by binding to DNA, thereby cleaving the DNA and inhibiting the growth of tumor cells, which is the basis for designing new and more efficient antitumor drugs, and their effectiveness depends on the mode and affinity of the binding [7–9]. Transition metal complexes that are capable of cleaving DNA under physiological conditions are of interest in the development of metal-based anticancer agents [10–12]. The clinical success of cisplatin and related platinum-based drugs, as anticancer agents that bind covalently to DNA, is severely affected by the serious side effects, general toxicity, and acquired drug resistance [13]. This is impetus to chemists to develop innovative strategies for the preparation of more effective, less toxic, target specific and preferably non-covalently bound anticancer drugs. In this regard, Cu(II) complexes are proving to be the most promising alternatives to cisplatin as anticancer drugs.

Planar heterocyclic base complexes have been at the forefront of these investigations because of their unusual electronic properties, diverse chemical reactivity, and peculiar structure, which results in non-covalent interactions with DNA. Copper, being a bio-essential transition metal ion, and its complexes, with tunable coordination geometries in a redox active environment, could find

Abbreviations: EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide); HOBt, hydroxybenzotriazole; TFA, trifluoroacetic acid; His-trp, histidyl-tryptophan; His-tyr, histidyl-tyrosine; phen, 1,10-phenathroline; CT-DNA, calf-thymus DNA; Tris, Tris(hydroxymethyl)aminomethane; EB, ethidium bromide; UV–Vis, ultraviolet–visible; DMSO, dimethyl sulfoxide; **1**, [Cu(II)(boc(tos)-his-trp-ome)(phen)]$(ClO_4)_2$; **2**, [Cu(II)(boc(tos)-his-tyr-ome)(phen)]$(ClO_4)_2$.

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better applications at the cellular level. Amino acids/peptides are the basic structural units of proteins that recognize a specific base sequence of DNA. An amino acid with a side chain aromatic ring, e.g. phenylalanine, tryptophan, tyrosine, etc., contributes mainly to the stabilization of proteins through hydrophobic interactions and the formation of hydrophilic environments [14,15].

In earlier publication [16], we isolated copper complexes of histidine containing dipeptides with aliphatic side chains, i.e. $[Cu^{II}(HisLeu)(phen)]^+$ and $[Cu^{II}(HisSer)(phen)]^+$, and studied their DNA binding and cleavage properties. Since aromatic moieties are known to play an important role in enhancing DNA binding and cleavage activity, we extended our study to dipeptides with aromatic side chains. Here we report the synthesis, characterization, DNA binding, hydrolytic and oxidative DNA cleavage activity of two new complexes, [Cu(II)(boc(tos)-his-trp-ome)(phen)]$(ClO_4)_2$ (**1**) and [Cu(II)(boc(tos)-his-tyr-ome)(phen)]$(ClO_4)_2$ (**2**). Compared to aliphatic dipeptide complexes, the aromatic dipeptide complexes show better DNA binding and cleavage activity. This reveals the importance of aromatic moieties in DNA binding and cleavage.

2. Experimental

2.1. Materials and instruments

EDCI, HOBt, DIPEA, TFA, $LiOH{\cdot}H_2O$, boc(tos)-histidine, tryptophan methyl ester, tyrosine methyl ester, $Cu(OAc)_2{\cdot}H_2O$, acetic acid, EDTA, phen and ethidium bromide (EB) were obtained from Sigma (99.99% purity) USA and were of analar grade. The peptides (boc(tos)-his-trp-ome and boc(tos)-his-tyr-ome) were synthesized by the conventional solution phase method [17]. CT-DNA was obtained from Fluka (Switzerland). Supercoiled plasmid pUC19 DNA, agarose, Tris–HCl and Tris base were obtained from Bangalore Genei (India). Solvents (MeOH, CH_2Cl_2 and THF) were purchased from Merck, India and all the chemicals were used as supplied. The spectroscopic titrations were carried out in aerated buffer (5 mM Tris–HCl/50 mM NaCl, pH 7.5) at room temperature (r.t.).

Elemental analyses data were obtained from the microanalytical Heraeus Carlo Erba 1108 elemental analyzer. The copper content was determined on a Shimadzu AA-6300 atomic absorption spectrophotometer. The molar conductivity was measured on a Digisun digital conductivity bridge (model: DI-909) with a dip type cell. Infrared spectra were recorded on a Perkin Elmer FT-IR spectrometer, in KBr pellets in the 4000–400 cm^{-1} range. Magnetic moments of the complexes were recorded at room temperature on a Faraday balance (CAHN-7600) using $Hg[Co(NCS)_4]$ as the standard. Diamagnetic corrections were made using Pascal's constants [18]. ESI mass spectra of the complexes were recorded using a Quattro Lc (Micro Mass, Manchester, UK) triple quadruple mass spectrometer with MassLynx software. UV–Vis spectra of the complexes were recorded on a Jasco V-530 UV–Vis spectrophotometer using 1 cm quartz micro-cuvettes. TGA analyses were obtained by a METLER TOLEDO (TGA/SDTA 851^e) thermo analyzer at 25–300 °C, with a heating rate of 5 °C/min. The molecular modeling calculations were carried out with a semi-empirical PM3 Hamiltonian as implemented in the hyperchem 6.0 software program package. The DNA cleavage experiments conducted using Genei (India) gel-electrophoresis equipment. The gel pattern of the electrophoresis was photographed by the Alpha-Innotec gel documentation system (USA).

2.2. Synthesis of the peptides and Cu(II) complexes

2.2.1. boc(tos)his-trp-ome and boc(tos)his-tyr-ome

The dipeptides (boc(tos)-his-trp-ome and boc(tos)-his-tyr-ome) were synthesized by adopting EDCI and HOBt as coupling agents in the presence of DIPEA and with dry CH_2Cl_2 as the solvent.

[boc(tos)-his-trp-ome]: ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.40 (s, 9H), 2.34 (m, 3H), 2.93–3.90 (s, 7H), 4.40–4.95 (m, 2H), 7.10–7.85 (m, 10H), 8.03 (m, 2H), 8.05 (m, 1H), 10.10 (m, 1H); ESI-MS, *m*/*z*: 609.

[boc(tos)-his-tyr-ome]: ^{1}H NMR (400 MHz, $CDCl_3$), δ: 1.40 (s, 9H), 2.34 (m, 3H), 2.80–3.90 (s, 7H), 5.30 (m, 1H), 4.40–4.95 (m, 2H), 6.65–7.85 (m, 9H), 8.03 (m, 2H), 8.05 (m, 1H); ESI-MS, *m*/*z*: 586.

2.2.2. [Cu(II)(boc(tos)-his-trp-ome)(phen)]$(ClO_4)_2$ (**1**) and [Cu(II)(boc(tos)-his-tyr-ome)(phen)] $(ClO_4)_2$ (**2**)

To a methanolic solution of boc-(tos)his-trp-ome (0. 609 g, 1.0 mM) for **1** or boc-(tos)his-tyr-ome (0.586 g, 1.0 mM) for **2**, a methanolic solution of $Cu(OAc)_2{\cdot}H_2O$ (0.198 g, 1.0 mM) was added under stirring, followed by the addition of phen (0.198 g, 1.0 mM) in MeOH (5 mL). The reaction was continued for ca. 6 h at room temperature and then an aq. solution of $NaClO_4{\cdot}H_2O$ (0.36 g, 2.0 mM) was added. A yellow precipitate was isolated and it was filtered and washed with ether, and then dried.

***[1]**: Anal.* Calc. for $C_{42}H_{43}Cl_2CuN_7O_{15}S$: C, 47.94; H, 4.12; N, 9.32; Cu, 6.04. Found: C, 47.89; H, 4.10; N, 9.28; Cu, 6.00%. IR (KBr, cm^{-1}): 3340br, 2926m, 1711m, 1665s, 1585s, 1518s, 1428s, 1366m, 1222m, 1166s, 1107m, 1034m, 1010s, 847s, 818m, 722s, 681m, 568m, 429s. UV–Vis [λ nm; MeOH:H_2O (1:10)]: 550, 395, 269, 212. ESI-MS *m*/*z*: 853 $[M+H]^+$. MP: 250 °C. μ_{eff} = 1.78 BM. Λ_M [Ω^{-1} cm^2 M^{-1}, 10^{-3} M in MeOH:H_2O (1:10), 25 °C]: 120. Yield: ~85%.

***[2]**: Anal.* Calc. for $C_{40}H_{42}Cl_2CuN_6O_{16}S$: C, 46.67; H, 4.11; N, 8.16; Cu, 6.17. Found: C, 46.63; H, 4.08; N, 8.12; Cu, 6.10%. IR (KBr, cm^{-1}): 3349br, 2926s, 1665s, 1597m, 1502m, 1426s, 1365m, 1244m, 1176m, 1092m, 955m, 867s, 745s, 723m, 553m, 429s. UV–Vis [λ nm; MeOH:H_2O (1:10)]: 540, 394, 268, 204. ESI-MS *m*/*z*: 830 $[M+H]^+$. MP: 265 °C; μ_{eff} = 1.76 BM. Λ_M [Ω^{-1} cm^2 M^{-1}, 10^{-3} M in MeOH:H_2O (1:10), 25 °C]: 150. Yield: ~85%.

2.3. DNA binding

2.3.1. Preparation of stock solution

Concentrated CT-DNA stock solution was prepared in 5 mM Tris–HCl/50 mM NaCl in double distilled water at pH 7.5 and the concentration of the DNA solution was determined by UV absorbance at 260 nm (ε = 6600 M^{-1} cm^{-1}) [19]. A solution of CT-DNA in 5 mM Tris–HCl/50 mM NaCl (pH 7.5) gave a ratio of UV absorptions at 260 and 280 nm A_{260}/A_{280} of ca. 1.8–1.9, indicating that the DNA was sufficiently free of protein [20]. All stock solutions were stored at 4 °C and were used within 4 days. The concentration of EB was determined spectrophotometrically at 480 nm (ε = 5680 M^{-1} cm^{-1}) [21].

2.3.2. Thermal denaturation (T_m) studies

Thermal denaturation studies were performed on a Shimadzu 160A spectrophotometer equipped with a thermostatic cell holder. The T_m value of CT-DNA was determined in the absence and presence of Cu(II) complexes by keeping the concentration of DNA and complexes **1** and **2** (30 μM) in a 1:1 ratio. The DNA samples were continuously heated at the rate of 1 °C/min temperature increase, while the absorption changes at 260 nm were monitored. The melting temperature (T_m), which is defined as the temperature where half of the total base pairs are unbound, was determined from the midpoint of the melting curves. ΔT_m values were calculated by subtracting T_m of free DNA from T_m of DNA interacting with the complex [22].

2.3.3. UV–Vis absorption spectroscopy

Absorption spectra were recorded on Jasco V-530 UV–Vis spectrophotometer using 1 cm quartz micro-cuvettes. Absorption titrations were performed by keeping the concentration of the