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Synthesis, crystal structures, DNA binding and cleavage activity of L-glutamine copper(II) complexes of heterocyclic bases

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ABSTRACT

Ternary L-glutamine (L-gln) copper(II) complexes [Cu(L-gln)(B)(H_2O)](X) (B = 2,2′-bipyridine (bpy), X = $0.5SO_4^{2-}$, **1**; B = 1,10-phenanthroline (phen), X = ClO_4^-, **2**) and [Cu(L-gln)(dpq)(ClO_4)] (**3**) (dpq, dipyridoquinoxaline) are prepared and characterized by physicochemical methods. The DNA binding and cleavage activity of the complexes have been studied. Complexes **1–3** are structurally characterized by X-ray crystallography. The complexes show distorted square pyramidal (4+1) CuN_3O_2 coordination geometry in which the N,O-donor amino acid and the N,N-donor heterocyclic base bind at the basal plane with a H_2O or perchlorate as the axial ligand. The crystal structures of the complexes exhibit chemically significant hydrogen bonding interactions besides showing coordination polymer formation. The complexes display a d–d electronic band in the range of 610–630 nm in aqueous-dimethylformamide (DMF) solution (9:1 v/v). The quasireversible cyclic voltammetric response observed near −0.1 V versus SCE in DMF–TBAP is assignable to the Cu(II)/Cu(I) couple. The binding affinity of the complexes to calf thymus (CT) DNA follows the order: **3** (dpq) > **2** (phen) ≫ **1** (bpy). Complexes **2** and **3** show DNA cleavage activity in dark in the presence of 3-mercaptopropionic acid (MPA) as a reducing agent *via* a mechanistic pathway forming hydroxyl radical as the reactive species. The dpq complex **3** shows efficient photo-induced DNA cleavage activity on irradiation with a monochromatic UV light of 365 nm in absence of any external reagent. The cleavage efficiency of the DNA minor groove binding complexes follows the order: **3** > **2** ≫ **1**. The dpq complex exhibits photocleavage of DNA on irradiation with visible light of 647.1 nm. Mechanistic data on the photo-induced DNA cleavage reactions reveal the involvement of singlet oxygen ($^1O_2$) as the reactive species in a type-II pathway.

1. Introduction

Transition metal complexes with their efficient DNA binding and cleavage properties under physiological conditions have found wide applications in nucleic acids chemistry [1–15]. The use of such complexes in footprinting studies, as sequence specific DNA binding agents, as diagnostic agents in medicinal applications and for genomic research has generated current interests to develop this chemistry further. The DNA cleavage reactions are generally targeted towards different constituents of DNA, viz. the heterocyclic bases, deoxyribose sugar moiety and phosphodiester linkage. The oxidative DNA cleavage involves in the nucleobase oxidation and/or degradation of the sugar moiety by abstraction of sugar hydrogen atom(s), while the hydrolytic cleavage of DNA takes place due to hydrolysis of the phosphodiester bond. Among different modes of DNA cleavage, oxidative cleavage of DNA on irradiation with visible light is of our interest for potential applications of such compounds in the chemistry of photodynamic therapy (PDT) of cancer [16–25].

Porphyrin and phthalocyanine-based organic dyes are known to cause oxidative cleavage of DNA on photo-irradiation in red light [16–18]. Such compounds are used as potential drugs in PDT [16]. Photofrin®, the FDA approved PDT drug, is a hematoporphyrin species. Transition metal complexes showing light-induced DNA cleavage activity are primarily limited to the ruthenium and rhodium complexes of polypyridyl bases for their known photophysical properties [21–25]. Recently, a platinum(IV) complex as a potential *cis*-platin alternative is reported to show photo-cytotoxic activity [26]. The chemistry of 3d-metal complexes showing DNA photocleavage activity in visible light is less explored [27–33]. We are interested to study the DNA binding and photo-induced DNA cleavage activity of bio-essential α-amino acid copper(II) complexes. Our recent reports have shown that ternary copper(II) complexes of the type "A-Cu-B", where the amino acid like L-methionine, L-lysine or L-arginine (A) and heterocyclic DNA-binding phenanthroline bases (B) are covalently linked to the metal ion, show efficient red light-induced DNA cleavage activity in the PDT window of 600–800 nm [34–38]. In contrast, organic conjugates

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of the type "A–B" in which an amino acid moiety (A) is covalently linked to a photoactive DNA intercalator (B) are known to show DNA cleavage activity only in UV light [39–41].

The present work stems from our interests to develop this chemistry further by synthesizing new ternary copper(II) complexes of α-amino acid L-glutamine and N,N-donor heterocyclic bases. This amino acid with its terminal –C(=O)–NH_2 group has the potential to form significant hydrogen bonding interactions with the double-stranded (ds) DNA and could show good DNA-binding propensity. Herein we report the synthesis, crystal structures, DNA-binding and oxidative DNA cleavage activity of ternary L-glutamine (L-gln) copper(II) complexes [Cu(L-gln)(B)(H_2O)](X) (B = 2,2′-bipyridine (bpy), X = $0.5SO_4^{2-}$, **1**; B = 1,10-phenanthroline (phen), X = ClO_4^-, **2**) and [Cu(L-gln)(dpq)(ClO_4)] (**3**) (dpq, dipyridoquinoxaline) (Scheme 1). The significant result of this study is the metal-assisted red light-induced DNA cleavage activity of complex **3**.

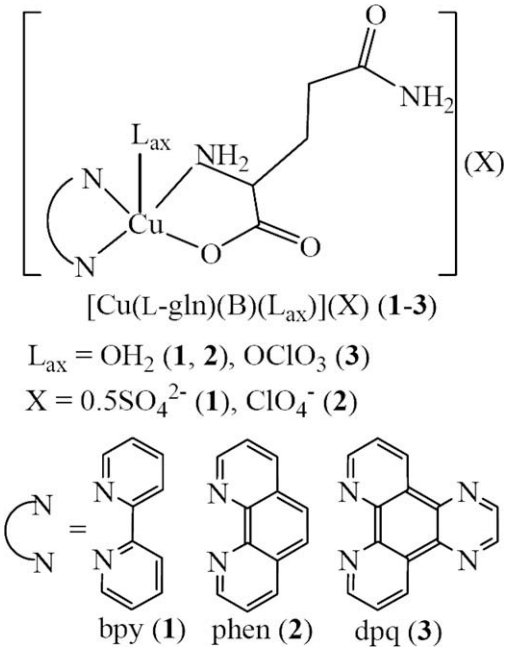
2. Experimental

2.1. Materials

The reagents and chemicals of analytical grade were procured from commercial sources. Solvents used for electrochemical and spectroscopic studies were purified by standard procedures [42]. Supercoiled pUC19 (CsCl purified) DNA was purchased from Bangalore Genei (India). Agarose (molecular biology grade), distamycin, catalase, superoxide dismutase (SOD), ethidium bromide (EB) and calf thymus (CT) DNA were purchased from Sigma (USA). Tris–HCl buffer solution was prepared using deionized and sonicated triple distilled water. Dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline (dpq) was prepared following a literature procedure [43].

2.2. General methods

Elemental analyses were done using a Thermo Finnigan Flash EA 1112 CHNSO analyzer. Infrared, absorption and fluorescence spectra were recorded on Perkin–Elmer Lambda 35, Perkin–Elmer Spectrum one 55 and Perkin–Elmer LS 50B spectrophotometers, respectively. Molar conductivity measurements were performed using a Control Dynamics (India) conductivity meter. Room temperature magnetic susceptibility data were obtained from a George Associates Inc. Lewis-coil force magnetometer using Hg[Co(NCS)$_4$] as a standard. Experimental susceptibility data were corrected for diamagnetic contributions [44]. Cyclic voltammetric measurements were made at 25 °C on a EG&G PAR Model 253 VersaStat potentiostat/galvanostat with electrochemical analysis software 270 using a three electrode set-up comprising of a glassy carbon working, platinum wire auxiliary and a saturated calomel reference (SCE) electrode. Tetrabutylammonium perchlorate (TBAP, 0.1 M) was used as a supporting electrolyte in DMF. The electrochemical data were uncorrected for junction potentials.



Scheme 1. Complexes **1**–**3** and the heterocyclic bases.

2.3. Synthesis of [Cu(L-gln)(B)(H_2O)](X) (B = bpy, X = $0.5SO_4^{2-}$, **1**; B = phen, X = ClO_4^-, **2**) and [Cu(L-gln)(dpq)(ClO_4)] (**3**)

The complexes were prepared by a general synthetic method in which an aqueous solution of $CuSO_4 \cdot 5H_2O$ (0.25 g, 1.0 mmol for **1**) or $Cu(ClO_4)_2 \cdot 6H_2O$ (0.37 g, 1.0 mmol for **2**, **3**) was initially reacted with an aqueous solution of L-glutamine (0.16 g, 1.1 mmol) treated with NaOH (0.040 g, 1.0 mmol), followed by slow addition of a methanol solution of the heterocyclic base [0.15 g, bpy, **1**; 0.18 g, phen, **2**; 0.22 g, dpq, **3** (0.9 mmol)]. The reaction mixture was stirred at 40 °C for 2 h and filtered. The filtrate on slow evaporation gave single crystals suitable for X-ray diffraction. The crystals were isolated and washed with aqueous methanol (1:1 v/v) before drying over P_4O_{10} (Yield: ~85%). For **1**, $C_{15}H_{19}CuN_4O_6S_{0.5}$: C, 41.78; H, 4.45; N, 13.01. Found: C, 41.47; H, 4.25; N, 13.12%. IR (KBr phase): 3363br, 3202br, 1658s, 1622s, 1497m, 1476m, 1445m, 1396m (SO_4^{2-}), 1319m, 1124s, 774m, 731m, 617m, 468w, 417w cm^{-1} (br, broad; w, weak; m, medium, s, strong; vs, very strong). Electronic spectrum in water [λ/nm (ε/M^{-1} cm^{-1})]: 237 (7950), 300 (8970), 310 (8250), 610 (50). Λ_M/S m^2 M^{-1} (in water, 25 °C) = 95. μ_{eff} (298 K): 1.78 μ_B. For **2**, $C_{17}H_{19}ClCuN_6O_7$: C, 42.23; H, 3.17; N, 15.55. Found: C, 42.17; H, 3.05; N, 15.42%. IR (KBr phase): 3525br, 3391br, 3070m, 1626m, 1608m, 1584s, 1555s, 1518m, 1446m, 1428s, 1340m, 1224m, 1147s, 1116s, 1088vs (ClO_4^-), 928m, 873m, 852s, 780w, 740m, 722s, 649w, 624s, 567w, 494m, 468m, 429w cm^{-1}. Electronic spectrum in H_2O [λ/nm (ε/M^{-1} cm^{-1})]: 273 (18060), 294 (5470), 622 (40). Λ_M/S m^2 M^{-1} (in H_2O, 25 °C) = 110. μ_{eff} (298 K): 1.74 μ_B. For **3**, $C_{19}H_{17}ClCuN_4O_8$: C, 40.32; H, 3.78; N, 11.06. Found: C, 40.17; H, 3.55; N, 10.94%. IR (KBr phase): 3697br, 3451br, 3296br, 3205br, 3054br, 1680s, 1643s, 1579m, 1486m, 1448w, 1409s, 1389s, 1339s, 1280w, 1230m, 1137s, 1083vs (ClO_4^-), 1060s (ClO_4^-), 1052s (ClO_4^-), 929m, 855m, 835s, 813m, 780m, 733s, 668m, 622s, 570s, 432m cm^{-1}. Electronic spectrum in H_2O [λ/nm (ε/M^{-1} cm^{-1})]: 258 (18500), 336 (1600), 628 (70). Λ_M/S m^2 M^{-1} (in H_2O, 25 °C) = 85. μ_{eff} (298 K): 1.72 μ_B.

2.4. Solubility and stability

The complexes showed good solubility in DMF, DMSO and water, less solubility in methanol and ethanol, and insolubility in hydrocarbons. They were found to be stable in the solid as well as in the solution phases. *Caution*! Perchlorate salts being potentially explosive, only small quantity was handled with care.

2.5. X-ray crystallographic procedures

The crystal structures of [Cu(L-gln)(bpy)(H_2O)]$(SO_4)_{1/2}$ (**1**), [Cu(L-gln)(phen)(H_2O)](ClO_4) (**2**) and [Cu(L-gln)(dpq)(ClO_4)] (**3**) were obtained by single crystal X-ray diffraction technique. Crystal mounting was done on glass fibers with epoxy cement. All geometric and intensity data were collected at room temperature using an